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A. R. CLAPHAM, H. GODWIN, W. O. JAMES

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THE MERTON CATALOGUE
A LIST OF THE CHROMOSOME NUMERALS OF SPECIES
OF BRITISH FLOWERING PLANTS¹

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INTRODUCTION

THE present list has a threefold object: First, to provide the systematist with a new instrument in classifying and determining his species of British flowering plants. Secondly, to provide the cytologist with the references to chromosome studies on these species, indicating to him at the same time the work that still remains to be done. Thirdly, to provide the naturalist with the means of understanding the conformities and unconformities of chromosome number which underlie the classification of these plants by their external form into species and larger groups.

We now know that the determination of a plant species by its morphological character alone breaks down in many genera. We realize this in more and more genera as the accuracy and extent of our knowledge increases. And we know that the chromosome number in these cases helps us to assign a plant to a precisely definable group, a group, moreover, which has the strictly practical sanction of fertility within itself and sterility with other groups known to have different numbers. This principle, which is always applied in scientific breeding practice, will now become applicable to systematic practice. It seems particularly appropriate that this attempt should be made first with the British Flora, which is so limited that it has become better known both morphologically and cytologically than any other in the world.

It has only just become possible to make a reasonably complete catalogue of this kind. The present list is therefore a first attempt to apply our knowledge of chromosome numbers systematically to the study of a local flora as extensive as that of the British Isles. It may be worth while therefore to explain the methods used in compiling the list, the reasons for them, and their inevitable shortcomings.

It will be seen that of the 526 genera of flowering plants found in Britain, 444 have been examined. Genera in which apomixis plays an important part have been marked specially, since apomictic species have not the same genetic meaning (or therefore practical value) as sexual species. The species included in the list are derived from the *London Catalogue* (which includes 2256 species), apart from a small number added by the various experts at Kew acknowledged below. Of these 1302 species have been counted. 413 species in the *London Catalogue* belong to

¹ Published under the auspices of the Association for the Study of Systematics in Relation to General Biology.

the great partially apomictic genera *Rosa*, *Rubus* and *Hieracium*. In these genera 99 species have been counted. In other genera 670 species remain unexamined. Their names are included in the list except in the especially numerous cases of *Juncus*, *Carex*, *Potamogeton* and *Euphrasia*.

For particulars of the place of origin of the material examined, reference must be made to the original papers. The inclusion of this information would too greatly increase the size of the list. A great deal of the material is of continental origin, and it will be necessary in due course to examine British material for comparative purposes. Ultimately we want to study the internal relations of each species from a comparison of its British and continental populations. It is particularly important for example to map the distribution of diploid and polyploid forms within the species. For this purpose pollen grain size may often be taken as a guide (cf. Darlington, 1937, for *Allium*, *Triticum* and *Rosa*).

Systematic conclusions can be derived from the numbers in this list in the light of what we know of the ways in which somatic chromosome numbers ($2n$) may change. These are, broadly speaking, of three kinds. First, there is polyploidy: a triploid ($3x$) arises by non-reduction in pollen or embryo-sac formation or a tetraploid ($4x$) arises by failure of a mitosis. A series of multiples of a basic number of chromosomes x may thus gradually develop. Odd multiples have no regular reduced number (n) and are sexually sterile. Secondly, there is a change in the number of this basic set. Any increase occurs by a process equivalent to fragmentation, a decrease by one equivalent to fusion. These processes are very common in some groups like *Crepis* and *Crocus*, rare in other groups like the Rosaceae and Gramineae. Thirdly, there is a simple reduplication of one or two members of the basic set. This change, unlike the others, produces a genetic change inherently, and when it occurs in a polyploid the product is called a secondary polyploid. Thus the Pomoideae with a basic set of 17 appear to be derived from the Roseae with a basic set of 7 in this way. Most monocotyledons and nearly all dicotyledons with haploid numbers of over 15 are polyploids of one kind or another. There is a fourth kind of change which is of no systematic importance, namely, the reduplication of small broken chromosomes which are usually inert and are irregularly inherited. These fragments where recognized are designated *ff*.

Finally, it must be noted that where more than one number has been given for one species this may sometimes be due to inaccurate naming of the material; but it is more likely to be due to the occurrence of two or more chromosome types within the species. The possibility of error in recent determinations of chromosome number is very slight, and all but 25 of the numbers given here have been determined since 1920.

The genetic and systematic interpretation of the chromosome numbers of species based on these principles is necessarily provisional. Only with the help and understanding of systematists will a rigorous interpretation of the processes underlying these changes in chromosome number be possible. It is to secure such help and understanding that I have prepared this list.

NOTE

The chromosome number given in all cases is $2n$.

References. Usually the latest is given, or, if not, the more important one. In some cases European works are preferred.

(A) refers to article on apomixis.

(G) refers to article on genetics.

(S) refers to article on systematics.

(T. 1927, T. 1931), etc., refers to Tischler's lists of chromosome numbers when the information is given there and not otherwise published. The basic number (x) is given after the genus. Many genera are dibasic. (apo) after a genus means that it is apomictic.

The nomenclature used is that of the *London Catalogue*, eleventh edition (1925). A certain number of corrections and additions have, however, been made at Kew. These bring the list more into line with recent work, but no attempt has been made to render it fully correct, either taxonomically or nomenclaturally. A new Students' Flora is in course of preparation, and it was considered unwise to attempt extensive corrections in the *London Catalogue* names pending its publication. Where the cytological author uses a different name this is given in square brackets, e.g. [as *triviale* Link.]. Where the eleventh edition of the *London Catalogue* uses a different name this is given in round brackets, e.g. (*Boreana* Jord.).

A or B after the number of a genus, e.g. 520 A *Pholiurus*, indicates that the genus has been subdivided and that *Pholiurus* does not appear in the *London Catalogue*.

Uncounted species are arranged alphabetically. The rest are arranged primarily by chromosome number, secondarily in alphabetic order. The numbers are taken in ascending order, except where there are two or more clear series, e.g. multiples of 7, 8, and 9 in *Veronica*.

ACKNOWLEDGEMENTS

I wish to thank the members of the staff of the Herbarium, Royal Botanic Gardens, Kew, who kindly checked this list and made a number of corrections and additions. I am also grateful to Mr E. M. Marsden-Jones of the Potterne Biological Station, who supplied many of the plants whose chromosomes I counted.

DICOTYLEDONES RANUNCULACEAE

1. CLEMATIS $x=8$

Vitalba L. 16 Maude unpub.

2. THALICTRUM (apo)* $x=7$ Schnarf 1929 (A)

alpinum L. 14 Kuhn 1930

minus L. 42 "

flavum L. 84 "

dunense Dum.

Kochii Fr.

majus Crantz

3. ANEMONE $x=7, 8$

apennina L. 16 Boecher 1932

Pulsatilla L. 32 Rosenthal 1936

ranunculoides L. 32 Langlet 1932

Nemorosa L. 32, 39 Boecher 1932

Moffett 1932

4. ADONIS $x=8$

annua L. 32 Langlet 1927

[as *autumnalis* L.]

5. MYOSURUS $x=8$

minus L. 16 Mann 1892

* Apomixis is known in *Thalictrum purpurescens* (Schnarf, 1929), so may occur in British species also.

6. *RANUNCULUS* (apo)
 $x=7, 8$

<i>acer</i> L.	14
<i>bulbosus</i> L.	16
<i>Ficaria</i> L.	16, 32 16+2 ff.
<i>hederaeus</i> L.	24
<i>ophioglossifolius</i> Vill.	c. 40
<i>sardous</i> Crantz	16
<i>scoticus</i> E. S. Marshall	16
<i>parviflorus</i> L.	28
<i>arvensis</i> L.	32
<i>auricomus</i> L.	32
<i>Baudottii</i> Godr.	48
<i>flabellatus</i> Desf.	32
<i>Flammula</i> L.	32
<i>Lenormandi</i> F. Schultz	32
<i>repens</i> L.	32
<i>reptans</i> L.	32
<i>peltatus</i> Schrank	32
<i>sceleratus</i> L.	32

Lingua L. 56 or 64?
128

<i>circinatus</i> Sibth.	..
<i>fluitans</i> Lam.	..
<i>heterophyllus</i> Weber	..
<i>lutarius</i> Bouvet	..
<i>pseudofluitans</i> Baker & Foggit	..
<i>sphaerosepium</i> Boiss. & Blanche	..
<i>trichophyllus</i> Chaix	..
<i>tripartitus</i> DC.	..

7. *CALTHA* $x=8$

<i>palustris</i> L.	32
<i>radicans</i> Forst.	48

8. *TROLLIUS* $x=8$

<i>europaeus</i> L.	16
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9. *HELLEBORUS* $x=8$

<i>foetidus</i> L.	32
<i>viridis</i> L.	32

10. *ERANTHIS* $x=8$

<i>hyemalis</i> Salisb.	16
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11. *AQUILEGIA* $x=7$

<i>vulgaris</i> L.	14
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12. *DELPHINIUM* $x=7, 8$

<i>Ajacis</i> L.	16
<i>Consolida</i> L.	16

13. *ACONITUM* $x=8$

<i>anglicum</i> Stapf.	32
------------------------	----

14. *ACTAEA* $x=8$

<i>spicata</i> L.	16
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15. *PAEONIA* $x=5$

<i>mascula</i> Mill.	10
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[as *corallina* Retz.] 10, 20

BERBERIDACEAE

16. *BERBERIS* $x=7$

<i>aquifolium</i> Pursh	28
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<i>vulgaris</i> L.	28
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17. *EPIMEDIUM* $x=6$

<i>alpinum</i> L.	12
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NYMPHAEACEAE

18. *NUPHAR* $x=17$

<i>lutea</i> Sibth. & Sm.	34
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<i>pumila</i> DC.	34
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19. *NYMPHAEA* $x=7, 8$

<i>alba</i> L.	84
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<i>occidentalis</i> Moss	112
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Gustafsson 1935 (C)

Boecher 1938

Marsden Jones &

Turrill 1935 (G)

Boecher 1938

Marsden Jones &

Turrill 1929 (G)

Boecher 1938

Larter 1932

Loschnigg 1926

Ribbands unpub.

Boecher 1932

Larter 1932

Langlet 1932

Larter 1932

Boecher 1938

Bruun 1932a

Boecher 1932

Larter 1932

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Boecher 1938

Larter 1932

Langlet 1936

Coonen 1939

Larter

Langlet 1932

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PAPAVERACEAE

20. *PAPAVER* $x=6, 7, 10, 11$

<i>hybridum</i> L.	14
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<i>Rhoeas</i> L.	14
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<i>somniferum</i> L.	20, 22
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<i>dubium</i> L.	28
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<i>Argemone</i> L.	42
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<i>Lecogii</i> Lamotte	12
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FUMARIACEAE

25. *CORYDALIS* $x=8$

<i>bulbosa</i> DC.	24
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<i>lutea</i> DC.	5b
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<i>claviculata</i> DC.	..
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34. DRABA x=8				sativum L.	16	Rarety 1932
incana L.	32	Heilborn 1927		latifolium L.	24	Wulf 1937 a
rupestris Br.	48	"		ruderalis L.	32	Jaretsky 1932
aizoides L.				Manton 1932
muralis L.		Draba L.	64	"
35. EROPHILA x=various				campestre Br.	..	"
verna Meyer	14, 28, 30,	Winge 1933 (S, G)		neglectum Thell.	..	"
	32, 36, 38,			47. THLASPI x=7		
	52, 58, 64,			alpestre L.	14	Chiarugi 1928
	94				14?	Manton 1932
	24, 40	Griesinger 1935		arvense L.	14	Chiarugi 1928
inflata Wats.	34, 54	Winge unpub.			c. 70	Manton 1932
		perfoliatum L.	..	Jaretsky 1932
36. COCHLEARIA x=7, 8				virens Jord.	..	"
groenlandica L.	14	Gairdner unpub.		48. IBERIS x=7, 8, 11		
alpina Wats.	28	Crane & Gairdner		amara L.	14	Resende 1937 a
		1923 (S, G)			14, 16	Jaretsky 1932
officinalis L.	28	Wulf 1937 a		49. TEESDALIA x=9		
Armoracia L.	32	Manton 1932		nudicaulis Br.	36	Jaretsky 1932
[as Armoracia lapathifolia						Manton 1932
Gilib.]				50. HUTCHINSIA x=6		
micacea E. S. Marshall	34-36	Crane & Gairdner		petraea R. Br.	12	Jaretsky 1929
		1923 (S, G)		51. ISATIS x=7		
danica L.	42	Wulf 1937 a		tinctoria L.	28	Jaretsky 1932
anglica L.	40-50	"				Manton 1932
37. HESPERIS x=6, 7				52. BUNIAS x=7		
matronalis L.	28?	Jaretsky 1928 a		orientalis L.	14	Resende 1937 a
38. SISYMBRIUM x=7				53. CRAMBE x=15		
altissimum L.	14	Jaretsky 1929		maritima L.	60	Jaretsky 1932
(parnonicum Jacq.)						Manton 1932
Irio L.	14	Manton 1932				Wulf 1937 a
officinale Scop.	14	Wulf 1937 b		54. CAKILE x=9		
orientale L.	14+ff?	Baez Major 1934		maritima Scop.	18	Jaretsky 1932
(Columnae Jacq.)						Manton 1932
Sophia L.	28	Manton 1932				Wulf 1937 a
		Jaretsky 1932		55. RAPHANUS x=9		
Alliaria Scop.	c. 42	"		maritimus Sm.	18	Manton 1932
[as A. officinalis Andrzej.]				Raphanistrum L.	18	Karpenchenko 1930
38A. ARABIDOPSIS x=5						(G, S)
Thaliana Schur.	10	Jaretsky 1928 a				
(Sisymbrium Thalianum Gay)						
39. ERISYMIUM x=7, 8						
Cheiranthoides L.	16	Manton 1932				
orientale Mill.				
40. CAMELINA x=10						
sativa Crantz	42	Jaretsky 1928 a				
	40	Manton 1932				
foetida Fr.	40	"				
41. SUBULARIA x=?						
aquatica L.				
42. BRASSICA						
x=8, 9, 10, 12						
nigra Koch	16	Nagai & Sasaoka				
		1930 a				
arvensis Kuntze	18	"				
[as Sinapis arvensis L.]						
oleracea L.	18	Lindenbein 1934				
Rapa L.	20	Catcheside 1934				
[as campestris L.]		Richhart 1937				
alba Boiss.	24	Nagai & Sasaoka				
[as Sinapis alba L.]		1930 a, b				
Wrightii O. E. Schulz	24	Nandi cit. Wright				
		1936				
Napus L.	38	Howard 1938				
Cheiranthus Vill.	48	Nandi cit. Wright				
		1936				
adpressa Boiss.				
Erucastrum Vill.				
monensis Huds.				
Rutabaga DC.				
43. DIPLOTAXIS x=7, 11						
tenusifolia DC.	22	Winge 1926				
	14, 56	Manton 1932				
muralis DC.	42	Maude unpub.				
44. CAPSELLA x=8						
Bursa-pastoris Medik.	32	Hill 1927				
		Shull 1937 (S, G)				
45. CORONOPUS x=8						
didymus Sm.	32	Jaretsky 1932				
		Manton 1932				
procumbens Gilib.	32	Jaretsky 1932				
		Manton 1932				
46. LEPIDIUM x=8						
Smithii Hook.	16	Manton 1932				
[as heterophyllum Benth.]						

VIOLA (cont.)

<i>montana</i> L.
<i>nana</i> Corbière
<i>obtusifolia</i> Jord.
<i>ruralis</i> Boreau

POLYGALACEAE

59. POLYGALA $x=?$			
<i>vulgaris</i> L.	48-56	Wulff 1938	
<i>amara</i> L.
(<i>amarella</i> Chodat)
<i>austriaca</i> Cr.
<i>calcarea</i> F. Schultz
<i>dubia</i> Belynk
<i>serpyllacea</i> Weihe
(<i>serpyllifolia</i> Hose)

FRANKENIACEAE

60. FRANKENIA $x=?$
<i>laevis</i> L.

CARYOPHYLLACEAE

61. DIANTHUS $x=15$			
<i>Armeria</i> L.	30	Rohweder 1937	
<i>deltoidea</i> L.	30		
<i>plumarius</i> L.	30, 60	Rohweder 1934	
<i>prolifer</i> L.	30, 60	Blackburn unpub.	
<i>gallicus</i> Pers.	45, 60	Andersson-Kottö & Gairdner 1931 (G)	
	60	Rohweder 1934	
	90	Gentscheff 1937	
<i>Caryophyllus</i> L.	30		
	60	Andersson-Kottö & Gairdner 1931 (G)	
	90	Rohweder 1934	
<i>caesi</i> Sm.	60		
	90	Andersson-Kottö & Gairdner 1931 (G)	
62. SAPONARIA $x=7, 15$			
<i>officinalis</i> L.	28	Blackburn & Boulton 1930	
<i>Vaccaria</i> L.	30	"	
[as <i>Vaccaria segetalis</i> Garcke]			
63. SILENE $x=12$			
<i>acaulis</i> L.	24	Blackburn 1928	
<i>Armeria</i> L.	24	"	
<i>comica</i> L.	24	"	
<i>conoidea</i> L.	24	"	
<i>dichotoma</i> Ehr.	24	"	
<i>gallica</i> L.	24	"	
<i>italica</i> Pers.	24	Blackburn 1929	
<i>maritima</i> With.	24	Blackburn 1928	
<i>noctiflora</i> L.	24	Heitz 1926	
<i>nutans</i> L.	24	Blackburn 1929	
<i>Otitis</i> Wibel	24	Griesinger 1937	
<i>anglica</i> L.
<i>vulgaris</i> Garcke
(<i>Cucubalus</i> Wibel)			
64. CUCUBALUS $x=12$			
<i>baccifer</i> L.	24	Blackburn 1928	
65. LYCHNIS $x=12$			
<i>alba</i> Mill.	24	Blackburn 1924	
<i>alpina</i> L.	24	Blackburn 1928	
[as <i>Viscaria alpina</i> G. Don]			
<i>dioica</i> Mill.	23-24	Blackburn 1924	
<i>Flos-Cuculi</i> L.	24	Blackburn 1928	
<i>Viscaria</i> L.	24	Rohweder (T. 1936)	
[as <i>Viscaria vulgaris</i> Trev.]			
<i>Githago</i> Scop.	48	Blackburn 1928	
[as <i>Agrostemma Githago</i> L.]			
66. HOLOSTEUM $x=10?$			
<i>umbellatum</i> L.	20	Blackburn (T. 1936)	
67. CERASTIUM $x=9?$			
<i>semidecandrum</i> L.	36	Rohweder unpub.	
<i>tetrandrum</i> Curt.	36	Wulff 1937a	
<i>arvense</i> L.	72	Rohweder unpub.	
<i>viscosum</i> L.	72	"	
[as <i>glomeratum</i> Thuill.]			
<i>vulgatum</i> L.	144	"	
[as <i>triviale</i> Link.]			
<i>alpinum</i> L.
<i>arcticum</i> Lange
<i>cerastoides</i> Britton
<i>pumilum</i> Curt.
<i>subtetrandrum</i> Murb.

68. MOENCHIA $x=9?$			
<i>erecta</i> Gaertn.	36	Blackburn (T. 1936)	

69. STELLARIA $x=?$			
<i>neglecta</i> Weihe	22	Peterson 1936	
<i>uliginosa</i> Murr.	24	" 1936	
<i>graminea</i> L.	26	"	
<i>Holostea</i> L.	26	" 1936	
<i>nemorum</i> L.	26	Rohweder unpub.	
<i>media</i> Vill.	40, 42, 44	Peterson 1936	
<i>aquatica</i> Scop.
<i>glauca</i> With.
<i>pallida</i> Piré
(<i>Boreana</i> Jord.)

70. ARENARIA $x=?$			
<i>trinervia</i> L.	24	Rohweder unpub.	
[as <i>Moehringia trinervia</i> Clairv.]			
<i>serpyllifolia</i> L.	40	"	
	20, 40	Griesinger 1937	
<i>peplodes</i> L.	48, 64	Rohweder unpub.	
<i>verna</i> L.	120?	"	
[as <i>Alsine vernea</i> Wahlenb.]			

<i>ciliata</i> L.
<i>gothica</i> Fr.
<i>leptoclados</i> Guss.
<i>norvegica</i> Gunn.
<i>rubella</i> Hook.
<i>sedoides</i> Druce
<i>temuifolia</i> L.
<i>uliginosa</i> Schleich.

71. SAGINA $x=?$			
<i>maritima</i> G. Don	22-24	Wulff 1937a	
	28	Blackburn (T. 1938)	
<i>nodosa</i> Fenzl.	20-24	Wulff 1937b	
	56	Blackburn (T. 1938)	
	22	"	

<i>procumbens</i> L.
<i>apetala</i> Ard.
<i>Boydii</i> F. B. White
<i>ciliata</i> Fr.
<i>filicaulis</i> Jord.
<i>Linnaei</i> Presl
<i>nivalis</i> Fr.
<i>Reuteri</i> Boiss.
<i>scotica</i> Druce
<i>subulata</i> Presl

72. SPERGULA $x=?$			
<i>sativa</i> Boenn.
<i>vulgaris</i> Boenn.

73. SPERGULARIA $x=9$			
<i>marginata</i> Kittel.	18	Wulff 1937a	
<i>salina</i> Presl	36	"	
<i>campestris</i> Willk. & Lange
<i>rubra</i> Presl
<i>rupicola</i> Lebel

74. POLYCARPON $x=?$			
<i>tetraphyllum</i> L.

PORTULACACEAE

75. CLAYTONIA $x=6$			
<i>alsinoides</i> Sims
<i>perfoliata</i> Donn
76. MONTIA $x=?$			
<i>fontana</i> L.

TAMARICACEAE

77. TAMARIX $x=?$			
<i>gallica</i> L.

ELATINACEAE

78. ELATINE $x=?$			
<i>Hydropiper</i> L.	c. 40	Frisendahl 1927	
<i>hexandra</i> DC.

HYPERICACEAE

79. HYPERICUM $x=8, 9, 10$			
<i>dubium</i> Leers.	16	Nielsen 1924	
[as <i>H. maculatum</i> Crantz]			
<i>humifusum</i> L.	16	Winge 1925	
<i>montanum</i> L.	16	Nielsen 1924	
<i>tetrapterum</i> Fr.	16	Winge 1925	
<i>perforatum</i> L.	32	Nielsen 1924	
<i>hirsutum</i> L.	18	"	

HYPERICUM (cont.)

<i>pulchrum</i> L.	18	Chattaway 1926
<i>calycinum</i> L.	20	"
<i>Androsaemum</i> L.	40	Nielsen 1924
<i>hircinum</i> L.	40	"
<i>Desetangii</i> Lam.
<i>elatum</i> Ait.
<i>elodes</i> L.
<i>linariifolium</i> Vahl
<i>undulatum</i> Schonsb.

MALVACEAE

80. <i>ALTHAEA</i> $\alpha = ?$		
<i>officinalis</i> L.	42	Skovsted 1935
	c. 42	Wulff 1937 a
<i>hirsuta</i> L.
81. <i>LAVATERA</i> $\alpha = ?$		
<i>arborescens</i> L.	36	Davie 1933
	36, 42	Skovsted 1935
<i>cretica</i> L.	40-44	Davie 1933
	c. 112	Skovsted 1935
82. <i>MALVA</i> $\alpha = ?$		
<i>rotundifolia</i> L.	40-60	Kristofferson 1926
[as <i>pusilla</i> Sm.]		(G)
<i>neglecta</i> Waltr.	42	Svensson-Stenar
[as <i>rotundifolia</i> L.]		1925
<i>moschata</i> L.	42	Davie 1933
<i>nicaeensis</i> All.	c. 64	Skovsted 1935
	42	Skovsted 1935
<i>parviflora</i> L.	42	Lilienfeld 1929
	40-44	Davie 1933
<i>sylvestris</i> L.	40	Latter 1932
	42	Davie 1933
<i>verticillata</i> L.	c. 84	Skovsted 1935

TILIACEAE

83. <i>TILIA</i> $\alpha = 41$		
<i>cordata</i> Mill.	82	Dermen 1932 a
<i>platyphyllos</i> Scop.	82	"
<i>vulgaris</i> Hayne	82	"

LINACEAE

84. <i>RADIOLA</i> $\alpha = ?$		
<i>linoides</i> Roth.
85. <i>LINUM</i> $\alpha = 8, 9, 15$		
<i>catharticum</i> L.	16	de Vilmorin &
		Simonet 1927
<i>anglicum</i> Mill.	18	Dillman 1933
[as <i>perenne</i> auctt. Brit.]		
<i>bienne</i> Mill.	30 (32)	Simonet 1929
[as <i>angustifolium</i> Huds.]		
<i>ustatissimum</i> L.	30	Kappert 1933 (G)
		Dillman 1933

GERANIACEAE

86. <i>GERANIUM</i>		
$\alpha = 9, 11, 12, 13, 14$		
<i>columbinum</i> L.	18	Gauger 1937
		Warburg 1938 a
<i>lucidum</i> L.	20	"
<i>dissectum</i> L.	22	Gauger 1937
		Warburg 1938 a
<i>pratense</i> L.	24	Tjebbes 1928
	28	Gauger 1937
		Warburg 1938 a
<i>pyrenaicum</i> Burm. f.	24?	Heitz 1926
	26	Gauger 1937
	28	Warburg 1938 a
<i>sylvaticum</i> L.	24	Tjebbes 1928
	28	Gauger 1937
		Warburg 1938 a
<i>molle</i> L.	26	Gauger 1937
	34	Warburg 1938 a
<i>pusillum</i> L.	26	Gauger 1937
<i>rotundifolium</i> L.	26	Warburg 1938 a
<i>nodosum</i> L.	28	Gauger 1937
<i>phaeum</i> L.	28	Warburg 1938 a
<i>versicolor</i> L.	28	"
<i>purpureum</i> Vill.	32	"
<i>Robertianum</i> L.	56	Gauger 1937
<i>sanguineum</i> L.	84	"
		Sansome 1936 (G)
87. <i>ERODIUM</i> $\alpha = ?$		
<i>moschatum</i> L'Hérit.	20	Gauger 1937

<i>cicutarium</i> L'Hérit.	36 (38)	Heitz 1926
	36, 40	Gauger 1937
	20, 40	Warburg 1938 b
<i>glutinatum</i> Dum.
<i>Lebelii</i> Jord.
<i>maritimum</i> L'Hérit.
<i>neglectum</i> Bak. f.

88. *OXALIS* $\alpha = 5, 7$, etc.

<i>Acetosella</i> L.	22-24	Heitz 1927
<i>stricta</i> L.	24	Wulff 1937 b
<i>corniculata</i> L.

89. *IMPATIENS*

$\alpha = 7, 8, 9, 10, 12$		
<i>capensis</i> Meerb.	20	F. H. Smith 1934
[as <i>biflora</i> Walt.]		
<i>Noli-tangere</i> L.	20	Winge 1925
<i>parviflora</i> DC.	24	Wulff 1934
<i>glandulifera</i> Royle	26	Heitz & Resende
		1936

AQUIFOLIACEAE

90. <i>ILEX</i> $\alpha = ?$		
<i>Aquifolium</i> L.	40	Maude unpub.

CELASTRACEAE

91. <i>EUONYMUS</i> $\alpha = ?$		
<i>europaeus</i> L.	64	Wulff 1937 b

RHAMNACEAE

92. <i>RHAMNUS</i> $\alpha = ?$		
<i>catharticus</i> L.
<i>Frangula</i> L.

ACERACEAE

93. <i>ACER</i> $\alpha = 13$		
<i>campestre</i> L.	26	Foster 1933
<i>pseudo-Platanus</i> L.	52	"

LEGUMINOSAE

94. <i>LUPINUS</i> $\alpha = ?$		
<i>nootkatensis</i> Donn	48	Maude unpub.
95. <i>GENISTA</i> $\alpha = 6$		
<i>pilosa</i> L.	24	Tscheckow 1931
<i>anglica</i> L.	42	Maude unpub.
<i>tinctoria</i> L.	48	Tscheckow 1931
96. <i>ULEX</i> $\alpha = 8$		
<i>minor</i> Roth	64	Tscheckow 1931
<i>europaeus</i> L.	96	"
<i>Gallii</i> Planch.
97. <i>CYTISUS</i> $\alpha = 12$		
<i>scoparius</i> Link	46	Maude unpub.
98. <i>ONONIS</i> $\alpha = 8$		
<i>repens</i> Linn. sp. pl.	32	Tscheckow 1933
(<i>arvensis</i> Linn. syst.)		
<i>spinosa</i> L.	32	"
<i>reclinata</i> L.	64	Tscheckow 1932
	60	Senn 1938
99. <i>TRIGONELLA</i> $\alpha = 7, 8$		
<i>ornithopodioides</i> DC.
100. <i>MEDICAGO</i> $\alpha = 8$		
<i>arabica</i> Huds.	16	Tscheckow 1933
<i>denticulata</i> Willd.	16	Ghimpu 1929
<i>lappacea</i> Desr.	16	Tscheckow 1933
<i>lupulina</i> L.	16	Ghimpu 1929
<i>minima</i> Desr.	16	Ghimpu 1929
<i>falcata</i> L.	32	Tscheckow 1933
<i>sativa</i> L.	32	Senn 1938
\times <i>varia</i> Martyn
(<i>sylvestris</i> Fr.)		
101. <i>MELILOTUS</i> $\alpha = 8$		
<i>alba</i> Desr.	16	Tscheckow 1933
		D. C. Cooper, 1933
<i>indica</i> Ail.	16	Clarke 1934
		Tscheckow 1933
<i>officinalis</i> Lam.	16	Clarke 1934
<i>altissima</i> Thuill.	..	Tscheckow 1933

102. TRIFOLIUM $x=7, 8$

<i>arvense</i> L.	14	Bleier 1925
<i>filiforme</i> L.	14	Karpechenko 1925
<i>incarnatum</i> L.	32	Wexelsen 1928
<i>pratense</i> L.	14	"
<i>procumbens</i> L.	14	Kawakami 1930
<i>fragiferum</i> L.	16	Williams 1935 (G)
		Karpechenko 1925
		Bleier 1925
		Karpechenko 1925
		Wulff 1937 a
<i>glomeratum</i> L.	16	Wexelsen 1928
<i>hybridum</i> L.	16	Kawakami 1930
<i>ochroleucon</i> Huds.	16	Bleier 1925
<i>resupinatum</i> L.	16	"
		Karpechenko 1925
<i>scabrum</i> L.	16	"
<i>squamosum</i> L.	16	"
<i>subterraneum</i> L.	16	Wexelsen 1928
<i>repens</i> L.	32	Senn 1938
<i>medium</i> L.	c. 96-98	Bleier 1925
<i>agrarium</i> L.
<i>Bocconi</i> Savi
<i>dubium</i> Sibth.
<i>Molineri</i> Balb.
<i>stellatum</i> L.
<i>striatum</i> L.
<i>suffocatum</i> L.

103. ANTHYLLIS $x=6, 7, 8$

<i>Vulneraria</i> L.	12	Tschechow & Kartashowa 1932
<i>maritima</i> Schweig
104. LOTUS $x=6$		
<i>angustissimus</i> L.	12, 24	Tschechow & Kartashowa 1932
<i>tenius</i> Waldst. & Kit.	12	"
[as <i>L. corniculatus</i> var. <i>tenius</i>]		
<i>uliginosus</i> Schkuhr	12	Dawson unpub.
<i>corniculatus</i> L.	24	"
<i>hispidus</i> Desf.	24	Tschechow & Kartashowa 1932

105. ASTRAGALUS $x=8$

<i>danicus</i> Retz.	16	Tschechow 1935
<i>glycyphyllos</i> Link	16	"
<i>alpinus</i> L.	c. 56	"

106. OXYTROPIS $x=8$

<i>uralensis</i> DC.	16	Tschechow 1935
<i>campestris</i> DC.

107. CORONILLA $x=6$

<i>varia</i> L.	12	Romanenko 1937
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108. ORNITHOPUS $x=7$

<i>perpusillus</i> L.	14	Maude unpub.
<i>pinnatus</i> Druce

109. HIPPOCREPIS $x=7$

<i>comosa</i> L.	28	Maude unpub.
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110. ONOBRYCHIS $x=7$

<i>viciifolia</i> Scop.	28	Hrubý unpub.
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111. VICIA $x=6, 7$

<i>angustifolia</i> L.	12	Heitz 1931 a
<i>hybrida</i> L.	12	Heitz 1931 b
<i>lathyroides</i> L.	12	"
<i>Orobis</i> DC.	12	"
<i>sativa</i> L.	12	Helm 1934
<i>Cracca</i> L.	14	Kawakami 1930
	12, 14	Sweshnikowa
		1929 (G, S)
		Senn 1938
<i>bithynica</i> L.	14	Sweshnikowa
		(1927 (G, S))
<i>gracilis</i> Lois.	14	"
<i>hirsuta</i> Gray	14	Kawakami 1930
<i>sepium</i> L.	14	Sweshnikowa 1927
<i>sylvatica</i> L.	14	"
<i>tetrasperma</i> Moench	14	"
<i>villosa</i> Roth	14	"
<i>laevigata</i> Sm.

112. LATHYRUS $x=7$

<i>Aphaca</i> L.	14	Senn 1938
<i>hirsutus</i> L.	14	"
<i>latifolius</i> L.	14	"
<i>maritimus</i> Bigel.	14	Simonet 1932 b
<i>montanus</i> Bernh.	14	Wulff 1937 a
		Wulff 1938

niger Bernh.

	14	Corti 1931
<i>Nissolia</i> L.	14	Senn 1938
<i>palustris</i> L.	14	"
<i>pratensis</i> L.	14	Melderis & Viksne
		1931
<i>sphaericus</i> Retz.	14	Senn 1938
<i>syloestris</i> L.	14	"
<i>tuberosus</i> L.	14	Fisk 1931

ROSACEAE

113. PRUNUS $x=8$

<i>avium</i> L.	16	Darlington 1928 (S, G)
		Crane & Lawrence
		1929 (G)
<i>spinosa</i> L.	16, 24, 32,	Darlington 1930 (G)
	40, 48	Mather 1937
<i>Cerasus</i> L.	32	Darlington 1928 (S, G)
		Crane & Lawrence
		1929 (G)
<i>Padus</i> L.	32	Sax 1931
<i>domestica</i> L.	48	Crane & Lawrence
		1929 (G)
		Darlington 1930 (G)
		Mather 1937
<i>insititia</i> L.	48	"

114. SPIRAEA $x=?$

<i>Filipendula</i> L.	15	Maude unpub.
[as <i>Filipendula hexa-</i>	14	Wulff 1938
<i>petala</i> Gil.]		
<i>Ulmaria</i> L.	14	Wulff 1938
	16	Ruesson 1938
<i>salicifolia</i> L.	36	Sax 1933

115. RUBUS $x=7$ (apo)

		Lidforss 1914 (G)
		Gustafsson 1930
		(G), 1935 (A)
		Crane & Lawrence
		1934
<i>Idaeus</i> L.	14	Crane & Darlington
		1927 (G, S)
		Crane & Lawrence
		1931 (G)
<i>rusticanus</i> Merc.	14	Crane & Darlington
		1927 (G, S)
<i>macrothyrsos</i> Lange	14, 28	Harrison unpub.
<i>thyrsoides</i> Wimm.	21	Longley 1924
<i>affinis</i> Weihe & Nees	28	Gustafsson 1933
		Fabergé unpub.
		Harrison unpub.
<i>apiculatus</i> Weihe & Nees	28	"
<i>axillaris</i> P. J. Muell.	28	Fabergé unpub.
<i>Bellardii</i> Weihe & Nees	28	Harrison unpub.
<i>Bertramii</i> G. Braun	28	Datta 1932
<i>Bloxamii</i> Lees.	28	Fabergé unpub.
<i>Borreri</i> Bell-Salt.	28	Datta 1932
<i>caesius</i> L.	28	Harrison unpub.
<i>cardiophyllus</i> Lefv. & Muell.	28	"
<i>cenomanensis</i> Sudre	28	"
<i>chrysosylon</i> Rogers	28	Bart. & Ridd
<i>cissburiensis</i>	28	"
<i>cordifolius</i> Blox.	28	Fabergé unpub.
<i>cryptadenes</i> Sudre	28	Harrison unpub.
<i>discerptus</i> P. J. Muell.	28	"
<i>dumoniensis</i> Bab.	28	"
<i>formidabilis</i> Lefv. & Muell.	28	Fabergé unpub.
<i>fusco-ater</i> Weihe	28	"
<i>gratus</i> Focke	28	Harrison unpub.
<i>heterobelus</i> Sudre	28	"
<i>hirtus</i> Waldst. & Kit.	28	Fabergé unpub.
<i>horridisepalus</i> Sudre	28	Datta 1932
<i>imbricatus</i> Hort.	28	"
<i>incurvatus</i> Bab.	28	Harrison unpub.
<i>insularis</i> Aresch.	28	"
<i>Koehleri</i> Weihe	28	"
<i>largificus</i> W. Wats.	28	Fabergé unpub.
<i>lentiginosus</i> Lees	28	Harrison unpub.
<i>Leyanus</i> Rogers	28	Gustafsson 1932 b
<i>Lindebergii</i> P. J. Muell.	28	Datta 1932
<i>Lindleanus</i> Lees	28	Harrison unpub.
<i>longifolius</i> W. Wats.	28	Fabergé unpub.
<i>macrophyloides</i> Genev.	28	"
<i>melanoderms</i> Focke	28	Harrison unpub.
<i>mollissimus</i> Rogers	28	"
<i>Newbouldii</i> Bab.	28	Datta 1932
<i>nitidus</i> Weihe & Nees	28	Harrison unpub.
<i>ornatus</i> Sudre	28	Gustafsson 1933
<i>plicatus</i> Weihe & Nees	28	"
<i>polyanthemus</i> Lindeb.	28	"

RUBUS (cont.)

- pseudobifrons* Sudre 28 Harrison unpub.
Questieri Lefv. & Muell. 28 Fabergé unpub.
radula Whe. 28 " "
rhombifolius Whe. 28 " "
roseaceus Whe. & Nees 28 Harrison unpub.
rotundifolius Blox. 28 Fabergé unpub.
Salteri Bab. 28 Harrison unpub.
scaber Weihe & Nees 28 Fabergé unpub.
scamicus Aresch. 28 Harrison unpub.
Schlechtendahl Weihe 28 Fabergé unpub.
Schlechter Weihe 28 Harrison unpub.
Selmeri Lindeb. 28 Fabergé unpub.
Sprengelii Weihe 28 Harrison unpub.
suberectus Anderss. 28 Gustafsson 1933
sulcatus Vest. 28 Fabergé unpub.
tardus 28 Wm Watson
thyrsiger Bab. 28 Crane & Darlington 1927
thyrsiflorus 28 Weihe
uncinatus P. J. Muell. 28 Harrison unpub.
vallisparvus Sudre 28 " "
villicaulis Koehl. 28 Datta 1932
Winteri Focke 28 Fabergé unpub.
radula Weihe 28 " "
corylifolius Sm. 35 Datta 1932
infestus Weihe 28, 42 Datta 1932
nitidoides W. Wats. 28 Fabergé unpub.
obscurissimus 42 Harrison unpub.
var. pallidistaminus 28, 42 " "
Bloxamianus Coleman. 42 " "
rotundellus Sudre 42 " "
Chamaemorus L. 50 La Cour unpub.
and 84 London Catalogue
species not counted
116. DRYAS $x=?$
octopetala L. 18 Maude unpub.
117. GEUM $x=7$
rivale L. 42 Prywer 1932
urbanum L. 42 " "
118. FRAGARIA $x=7$
vesca L. 14 East 1934
moschata Duchesne 42 Schiemann 1937
[as F. elatior Ehrh.] (G)
Fedorova 1934
Schiemann 1937 (G)
Gustafsson 1935 (A)
119. POTENTILLA $x=7$ (apo)
argentea L. 14, 42, 56 Müntzing 1931 b
fruticosa L. 14 Sax 1931
rupestris L. 14 Popoff 1935
sterilis Garcke 14 Shimotomai 1929
[as fragarioides Vill.] 28 Wulff 1938
intermedia L. 28 Shimotomai 1930 a, b
reptans L. 28 Tischler 1929 b
Anserina L. 32 Popoff 1935
erecta Hampe 32 Forenbacher 1914
Crantzii G. Beck 42, 49 Müntzing 1931 b
recta L. 42 Shimotomai 1930 a, b
verna L. 28 Popoff 1935
norvegica L. 42, 84 Müntzing 1931 b
palustris Scop. " " " "
procumbens Sibth. " " " "
Sibbaldi Hall. f. " " " "
120. ALCHEMILLA $x=8$ (apo)
arvensis Scop. 48 Böös 1924
alpina L. 64 Strasburger 1904
[as A. grossidens Buser]
vulgaris L. (agg.) 64 Murbeck 1901
[as A. acutangula Buser]
11 species not counted.
121. AGRIMONIA $x=7$
Eupatoria L. 28 Maude unpub.
odorata Mill. " " " "
122. POTERIUM $x=?$
Sanguisorba L. 28 Maude unpub.
officinale A. Gray " " " "
polygamum Waldst. & Kit. " " " "
123. ROSA $x=7$ (apo)
arvensis Huds. 14 Gustafsson 1935 (A)
glauca Vill. 28 Hurst 1931 (G, S)
mollis Sm. 28 " "
omissa Déségl. 28, 56 Erlanson 1933
pomifera Herrm. 28, 35, 42 Harrison unpub.
rubella Sm. 28 Erlanson 1933
spinosissima L. 28 Harrison unpub.
agrestis Savi 35 Hurst 1931
canina L. 35 " "
coriifolia Fr. 35 Harrison unpub.
dumetorum Thuill. 35 Hurst 1931
elliptica Tausch. 35 " "
micrantha Sm. 35 Harrison unpub.
rubiginosa L. 35, 42 Hurst 1931
stylosa Desr. 35 " "
tomentella Lem. 35, 42 Harrison unpub.
tomentosa Sm. 35 Hurst 1931
25 species not counted.
124. SORBUS $x=17$ (apo)
Aria Crantz 34 Gustafsson 1935 (A)
aucuparia L. 34 Moffett 1931 a, b
domestica L. 34 Sax 1931
terminalis Crantz 34 Moffett 1931 a, b
minima Hedl. 51 Sax 1931
Mougeoti Soyer-Will. & Godr. 51 Liljefors 1934
fennica Fr. 68 " "
rupicola Hedl. 68 " "
arranensis Hedl. " " " "
latifolia Pers. " " " "
porrigens Hedl. " " " "
scandica Fr. " " " "
125. PYRUS $x=17$
communis L. 34 Moffett 1931 a, b
Malus L. 34, 51 Darlington & Moffett 1930
Crane & Lawrence 1930 (G)
cordata Desv. " " " "
germanica Hook. f. " " " "
126. CRATAEGUS $x=17$
monogyna Jacq. 34 Moffett 1931 a, b
Oxyacantha L. 34 " "
127. COTONEASTER $x=17$
microphylla Wallich 68 Moffett 1931 a, b
integerrima Medic. " " " "

SAXIFRAGACEAE

128. SAXIFRAGA $x=7, 8, 11, 13$
tridactylites L. 22 v. Drygalski 1935
atroides L. 26 Skovsted 1934 (S, G)
oppositifolia L. 26 Skovsted 1934
Geum L. 28 " "
nivalis L. 28 " "
stellaris L. 28 " "
umbrosa L. 28 Schürhoff 1925
caespitosa L. 32 Whyte 1930
granulata L. 56, 60, 63, Skovsted 1934
65
32 Schürhoff 1925
Whyte 1930
28-30 Schoennagel 1931
40, 47, 48, Skovsted 1934
49, 50, 51,
53, 54, 56,
57, 60
hypnoides L. c. 58 Marsden Jones & Turrill 1938 (G)
c. 44 Skovsted 1934
rosacea Moench 64 Marsden Jones & Turrill 1938 (G)
Philp 1934
Marsden Jones & Turrill 1934 (G, S)
Skovsted 1934
cervina L. c. 66
affinis D. Don " " " "

SAXIFRAGA (cont.)

<i>Drucei</i> E. S. Marshall
<i>Hirculus</i> L.
<i>hirsuta</i> L.
<i>hirta</i> Sm.
<i>incurvifolia</i> D. Don.
<i>leptophylla</i> D. Don.
<i>platypetala</i> Sm.
<i>rivularis</i> L.
<i>sponhemica</i> Gmel.
<i>Sternbergii</i> Willd.

129. CHRYSOSPLENIUM

<i>oppositifolium</i> L.	42	Schoennagel 1931
<i>alternifolium</i> L.	48	Skovsted 1934
130. PARNASSIA $x=9$		
<i>palustris</i> L.	18	Matsuura & Sutô 1935
131. RIBES $x=8$		
<i>alpinum</i> L.	16	Meurman 1925
<i>Grassularia</i> L.	16	Sax 1931
<i>nigrum</i> L.	16	Meurman 1928
<i>pubescens</i> Hartm.	16	Tischler 1927 b
<i>rubrum</i> L.	16	Meurman 1928

CRASSULACEAE

132. TILLAEA $x=?$		
<i>aquatica</i> L.
<i>muscosa</i> L.
133. COTYLEDON $x=?$		
<i>Umbilicus-Veneris</i> L.
134. SEDUM $x=8, 17$		
<i>Telephium</i> L.	22, 24, 28, 36, 50	Baldwin 1937
	48	Turesson 1938
<i>reflexum</i> L.	34, 68	Baldwin 1935
<i>acre</i> L.	48	Wulff 1937 b
<i>album</i> L.	64	Baldwin 1935
<i>anglicum</i> Huds.
<i>dasyphyllum</i> L.
<i>Forsterianum</i> Sm.
<i>Rosea</i> Scop.
<i>rupestre</i> L.
<i>sexangulare</i> L.
<i>villosum</i> L.
135. SEMPERVIVUM $x=?$		
<i>tectorum</i> L.

DROSERACEAE

136. DROSERA $x=10$		Rosenberg 1909 a
		(S)
<i>rotundifolia</i> L.	20	Nakajima 1933
<i>longifolia</i> L.	40	Behre 1929
<i>anglica</i> Huds.

HALORAGACEAE

137. HIPPURIS $x=?$		
<i>vulgaris</i> L.	32	Winge 1917
138. MYRIOPHYLLUM $x=?$		
<i>alternifolium</i> DC.
<i>spicatum</i> L.
<i>verticillatum</i> L.
139. CALLITRICHE $x=3, 5$		
<i>autumnalis</i> L.	6	Sokolovskaja 1932
<i>truncata</i> Guss.	6	Dodds (T. 1936)
<i>stagnalis</i> Scop.	10	Sokolovskaja 1932
<i>vernalis</i> Koch	20	"
<i>intermedia</i> Hoffm.
<i>obtusangula</i> Le Gall.
<i>polymorpha</i> Lönnr.

LYTHRACEAE

140. PEPLIS $x=?$		
<i>Portula</i> L.
141. LYTHRUM $x=5$		
<i>Hyssopifolia</i> L.	20	Tischler 1929 a
<i>Salicaria</i> L.	30	Takahashi cit.
		Kihara et al. 1931
	50	Tischler 1929 a
		Haldane 1936 (G)

ONAGRACEAE

142. EPILOBIUM $x=9$		
<i>collinum</i> Gmel.	36	Griesinger 1937
<i>montanum</i> L.	36	Håkansson 1924
		Schwemmle 1924
<i>parviflorum</i> Schreb.	36	Michaelis 1925
<i>roseum</i> Schreb.	36	Schwemmle 1924
<i>tetragonum</i> Curt.	36	Turesson 1938
<i>hirsutum</i> L.	54	Michaelis 1928
<i>adenocaulon</i> Hausskn.
<i>alsinifolium</i> Vill.
<i>anagallidifolium</i> Lam.
<i>Lamyi</i> F. Schultz
<i>lanceolatum</i> Seb. & Maur
<i>obscureum</i> Schreb.
<i>palustre</i> L.
142A. CHAMAENERION $x=9$		
<i>angustifolium</i> Schur	36	Johansen 1929 a, b
(<i>Epilobium angustifolium</i> L.)		
143. LUDWIGIA $x=?$		
<i>palustris</i> Elliott
144. OENOTHERA $x=7$		
<i>amnophila</i> Focke	14	Renner 1925 (G)
		Rudloff & Schmidt 1932
<i>biennis</i> L.	14	Wulff 1937 a
<i>Lamarckiana</i> Seringe	14	Darlington 1931
<i>stricta</i> Ledeb.
145. CIRCAEA $x=11$		
<i>alpina</i> L.	22	Uddling 1929
<i>intermedia</i> Ehrh.	22	"
<i>lutetiana</i> L.	22	"

CUCURBITACEAE

146. BRYONIA $x=10$	20	Resende 1937
<i>dioica</i> Jacq.		

UMBELLIFERAE

147. HYDROCOTYLE $x=?$		
<i>vulgaris</i> L.	64 or 66	Wanscher 1931
148. ERYNGIUM $x=7, 8$		
<i>campestre</i> L.	14	Tamamschjan 1933
<i>maritimum</i> L.	16	Wulff 1937 a
149. ASTRANTIA $x=7$		
<i>major</i> L.	14	Wanscher 1932
150. SANICULA $x=8$		
<i>europaea</i> L.	16	Wanscher 1931
151. DANÆA $x=?$		
<i>cornubiensis</i> Burnat
152. CONIUM $x=8, 11$		
<i>maculatum</i> L.	16	Nordheim 1930
	22	Wanscher 1932
153. SMYRNIUM $x=11$		
<i>Olusatrum</i> L.
154. BUPLEURUM $x=8, 11$		
<i>rotundifolium</i> L.	16	Schulz-Gaebel 1930
	22	Tamamschjan 1933
<i>tenuissimum</i> L.	16	Melderis 1930
		Wanscher 1933 a
		Wulff 1937 a
<i>falcatum</i> L.
<i>opacum</i> Lange
155. TRINIA $x=9$		
<i>glauca</i> Reichb. f.	18	Wanscher 1933 a
[as <i>vulgaris</i> DC.]		
156. APIUM $x=11$		
<i>graveolens</i> L.	22	Wanscher 1931
		Wulff 1937 a
<i>imundatum</i> Reichb. f.
<i>modiflorum</i> Reichb. f.
<i>repens</i> Reichb.
157. CICUTA $x=11$		
<i>virosa</i> L.	22	Melderis 1930

158. CARUM $x=10?$, 11
Carvi L. 20 Wanscher 1931
 22 Schulz-Gaebel 1930
Bulbocastanum Koch 22
segetum Benth. 22
verticillatum Koch 22
 158A. PETROSELINUM $x=?$
crispum Nym. 22
 (Carum Petroselinum Benth. & Hook. f.) 22
 159. SISON $x=?$
Amomum L. 22
 160. FALCARIA $x=?$
vulgaris Bernh. 22
 161. SIUM $x=10?$, 11?
latifolium L. 20 Wulff 1938
erectum Huds. 22
 162. AEGOPODIUM $x=11$
Podagraria L. 44 Melderis 1930
 163. PIMPINELLA $x=9$
Saxifraga L. 18 Schulz-Gaebel 1930
 18, 36 Håkansson 1933a
major Huds. 22
 [as magna L.] 22
 164. CONOPODIUM $x=?$
majus Loret 22
 165. MYRRHIS $x=11$
odorata Scop. 22 Marchal 1920
 166. CHAEROPHYLLUM $x=11$
aureum L. 22 Schulz-Gaebel 1930
temulum L. 22
 167. SCANDIX $x=8$
Pecten-Veneris L. 16 Wanscher 1931
 168. ANTHRISCUS $x=8$, 9
sylvestris Hoffm. 16 Wanscher 1931
Cerefolium Hoffm. 18 Tureson 1938
vulgaris Bernh. 18 Wanscher 1931
 169. SESELI $x=11$
Libanotis Koch 22 Wanscher 1932
 [as Libanotis montana Crantz] 22
 170. FOENICULUM $x=11$
vulgare Mill. 22 Ogawa 1929
 171. CRITHMUM $x=11$
maritimum L. 22 Wanscher 1932
 172. OENANTHE $x=11$
aquatica Poir. 22 Wulff 1938
Lachenalii Gmel. 22 Wulff 1937a
pimpinelloides L. 22 Wanscher 1931
crocata L. 22
fistulosa L. 22
fluviatilis Coleman 22
silaifolia Bieb. 22
 173. AETHUSA $x=10?$, 11?
Cynapium L. 20 Wanscher 1931
 22 Schulz-Gaebel 1930
 174. SILER $x=11$
trilobum Crantz 22 Wanscher 1932
 175. SILAUS $x=11$
flavescens Bernh. 22 Maude unpub.
 176. MEUM $x=11$
Athamanticum Jacq. 22 Wanscher 1931
 177. LIGUSTICUM $x=11$
scoticum L. 22 Wanscher 1932
 178. SELINUM $x=11$
Carvifolia L. 22 Schulz-Gaebel 1930
 179. ANGELICA $x=11$
sylvestris L. 22 Ogawa 1929
Archangelica L. 22 Wanscher 1931
 (180. Archangelica officinalis Hoffm.) 22
 181. PEUCEDANUM $x=11$
Ostruthium Koch 22 Wanscher 1931
palustre Moench 22 Schulz-Gaebel 1930
sativum Benth. & Hook. f. 22 Melderis 1930
officinale L. 22 Schulz-Gaebel 1930

182. HERACLEUM $x=11$
Sphondylium L. 22 Maude unpub.
 183. TORDYLIUM $x=11$
maximum L. 22 Tamamschjan 1933
 184. CORIANDRUM $x=11$
sativum L. 22 Wanscher 1932
 185. DAUCUS $x=11$
Carota L. 22 Melderis 1930
 18 gummiifer All. 18 Maude unpub.
 18 Lindenbein 1932
 186. CAUCALIS $x=?$
Anthriscus Huds. 16 Melderis 1930
 [as Torilis Anthriscus Gmel.] 20
daucoides L. 20 Wanscher 1932
latifolia L. 32?
arvensis Huds. 22
nodosa Scop. 22

ARALIACEAE

187. HEDERA $x=?$
Helix L. c. 44, 88 Oehm 1924
 [as colchica C. Koch] c. 120 Wanscher 1933a

CORNACEAE

188. CORNUS $x=9$, 11
sanguinea L. 22 Dermen 1932b
suecica L. 22

CAPRIFOLIACEAE

189. ADOXA $x=9$
Moschatellina L. 36 Geitler 1935c
 56 Matsura & Sutō 1935
 190. SAMBUCUS $x=9$
nigra L. 36 Sax & Kribs 1930
Ebulus L. 22
 191. VIBURNUM $x=9$
Lantana L. 18 Sax & Kribs 1930
Opulus L. 18
 192. LINNAEA $x=?$
borealis L. 22
 193. LONICERA $x=?$
Xylosteum L. 18-20 Feng 1934
Caprifolium L. 22
Periclymenum L. 22

RUBIACEAE

194. RUBIA $x=?$
peregrina L. 132? Fagerlind 1937b (S. G)
 Fagerlind 1934
 195. GALIUM $x=10$, 11, 12
Vaillantii DC. 20 Fagerlind 1934
Cruciata Scop. 22
Mollugo L. 22, 44 Fagerlind 1937b
uliginosum L. 22, 44 Fagerlind 1934
verum L. 22, 44, 66 Fagerlind 1937b
palustre L. 24 Fagerlind 1934
 66? Homeyer (T. 1936)
 95?, 100 Fagerlind 1937b
Aparine L. 44 Homeyer 1935
 64, 86 Fagerlind 1934
boreale L. 44
 66 Tureson 1938
saxatile L. 44 Fagerlind 1934
syloestris Poll. 44
tricornis Stokes 44 Fagerlind 1937b
anglicum Huds. 22
debile Desv. 22
erectum Huds. 22
 196. ASPERULA $x=11$
arvensis L. 22 Homeyer 1934
taurina L. 22 Fagerlind 1934
cynanchica L. 44
 22 Homeyer 1932
odorata L. 44 Fagerlind 1937b
 197. SHERARDIA $x=11$
arvensis L. 22 Fagerlind 1934

VALERIANACEAE

198. VALERIANA $x=7, 8$		
<i>officinalis</i> L.	14	Runquist 1937
	28	Meurman 1931
<i>dioica</i> L.	16	Meurman 1925
<i>sambucifolia</i> Mikan	56	Meurman 1931
<i>pyrenaica</i> L.
199. KENTRANTHUS		
$x=?$		
<i>Calcitrapa</i> Dufr.
<i>ruber</i> DC.
200. VALERIANELLA		
$x=7, 9$		
<i>dentata</i> Poll.	14	Elvers 1932
<i>ericaarpa</i> Desv.	14	"
<i>olitoria</i> Poll.	14	"
<i>rimosa</i> Bast.	14	"
<i>carinata</i> Lois.	18	"

DIPSACACEAE

201. DIPSACUS $x=9$		
<i>pilosus</i> L.	18	Kachidze 1929
<i>syvestris</i> Huds.	16?	Braun (T. 1936)
	18	Kachidze 1929
202. SCABIOSA $x=8, 9$		
<i>arvensis</i> L.	16	Jaeger 1934
[as <i>Knautia arvensis</i> Coult.]	20	Wulff 1938
<i>Columbata</i> L.	16	Risse 1928
		Kachidze 1929
<i>maritima</i> L.	16	Risse 1928
		Kachidze 1929
<i>Succisa</i> L.	20	Kachidze 1929
[as <i>Succisa pratensis</i> Moench]		

COMPOSITAE

203. EUPATORIUM		Gustafsson 1935 (A)
$x=10, 17$ (apo)		
<i>cannabinum</i> L.	20	Holmgren 1919
204. SOLIDAGO $x=?$		
<i>Virgaurea</i> L.
205. BELLIS $x=9$		
<i>perennis</i> L.	18	Blackburn 1934
206. ASTER $x=5, 9$		
<i>Tripolium</i> L.	18	Wulff 1937a
<i>Linosyris</i> Bernh.
<i>Novi-Belgii</i> L.
<i>salignus</i> Willd.
207. ERIGERON $x=9$ (apo)		
<i>borealis</i> Vierh.	18	Gustafsson 1935 (A)
(<i>alpinum</i> auctt. angl.)		Chiarugi 1927
<i>canadensis</i> L.	18	Okabe 1934
<i>acris</i> L.
208. FILAGO $x=7$		
<i>germanica</i> L.	28	Wulff 1937b
<i>apiculata</i> G. E. Smith
<i>gallica</i> L.
<i>minima</i> Fr.
<i>spatulata</i> Presl
209. ANTENNARIA		Gustafsson 1935 (A)
$x=7$ (apo)		
<i>dioica</i> Gaertn.	28	Bergman 1935a
210. ANAPHALIS $x=7$		
<i>margaritacea</i> Benth. & Hook. f.	28	Maude unpub.
211. GNAPHALIUM $x=7$		
<i>uliginosum</i> L.	14	Wulff 1938
<i>luteo-album</i> L.	14	"
<i>norvegicum</i> Gunn.
<i>supinum</i> L.
<i>sylvaticum</i> L.
<i>undulatum</i> L.
212. INULA $x=8$		
<i>britannica</i> L.	16, 24	Okabe 1937
[as subsp. <i>japonica</i>]		
<i>Helenium</i> L.	c. 20	Tongiorgi 1935
<i>squarrosa</i> Bernh.	32	"
[as <i>I. Conyza</i> DC.]		
<i>crithmoides</i> L.
<i>salicina</i> L.
213. PULICARIA $x=?$		
<i>vulgaris</i> Gaertn.	18	Wulff 1937b
<i>dysenterica</i> Gray	20	Rodolico 1933

214. BIDENS $x=12$		
<i>ternata</i> L.	24	Lewitzky 1934
<i>cripartita</i> L.	48	"
215. GALINSOGA $x=?$		
<i>parviflora</i> Cav.	36	Nawaschin (T. 1927a)
216. ACHILLEA $x=9$		
<i>Ptarmica</i> L.	18	Lewitzky 1934
<i>Millefolium</i> L.	36, 54	Turesson 1938
217. DIOTIS $x=?$		
<i>maritima</i> Cass.
218. ANTHEMIS $x=9$		
<i>arvensis</i> L.	18	Wulff 1937b
<i>Cotula</i> L.	18	"
<i>tinctoria</i> L.	18	Holmgren 1915
<i>nobilis</i> L.
219. CHRYSANTHEMUM		
$x=9$		
<i>segetum</i> L.	18	Tahara 1921
<i>Leucanthemum</i> L.	36	Shimotomai 1937
<i>Parthenium</i> Bernh.
220. <i>Matricaria</i> $x=9$		
<i>Chamomilla</i> L.	18	Maude unpub.
<i>inodora</i> L.	18	Hüser (T. 1936)
<i>suaveolens</i> Buch.
221. COTULA $x=10$		
<i>coronopifolia</i> L.	20	Wulff 1937b
222. TANACETUM $x=?$		
<i>vulgare</i> L.
223. ARTEMISIA $x=9$		
<i>Absinthium</i> L.	18	Weinadel-Liebau 1928
<i>campestris</i> L.	18	"
<i>maritima</i> L.	36	Erlandsson 1939
	18	Weinadel-Liebau 1928
<i>vulgaris</i> L.	18	Wulff 1937a
		Weinadel-Liebau 1928
<i>Stelleriana</i> Bess.
224. TUSSILAGO $x=?$		
<i>Farfara</i> L.	60	Langlet 1936
225. PETASITES $x=?$		
<i>fragrans</i> Presl	52	Maude unpub.
[as <i>P. officinalis</i> Moench]		
<i>ovatus</i> Hill	60	Langlet 1936
<i>albus</i> Gaertn.
226. DORONICUM $x=?$		
<i>Pardalianches</i> L.
<i>plantagineum</i> L.
227. SENECEO $x=10, 12$		
<i>squalidus</i> L.	20	Afzelius 1924
<i>Cineraria</i> DC.	40	"
<i>erucifolius</i> L.	40	"
<i>Jacobaea</i> L.	40	"
<i>paludosus</i> L.	40	"
<i>sylvaticus</i> L.	40	"
<i>viscosus</i> L.	40	"
<i>vulgaris</i> L.	40	"
<i>palustris</i> Hook.	48	"
<i>aquaticus</i> Hill
<i>erraticus</i> Bertol.
<i>integrifolius</i> Clairv.
<i>sarracenicus</i> L.
<i>spatulifolius</i> DC.
228. CARLINA $x=10$		
<i>vulgaris</i> L.	20	Lewitzky (T. 1936)
229. ARCTIUM $x=8$		
<i>majus</i> Bernh.	32	Sugiura 1931
(<i>Lappa</i> L.)	36	Nakajima 1936
<i>minus</i> Bernh.	32	Wulff 1937b
<i>pubens</i> Bab.
230. CARDUUS $x=8$		
<i>crispus</i> L.	16	Poddubnaja-Arnoldi 1927
<i>nutans</i> L.	16	Poddubnaja-Arnoldi 1931
<i>pycnocephalus</i> L.
<i>tenuiflorus</i> Curt.

231. CIRSIIUM (CNICUS)

 $x=17$

- acaule* Weber 34 Wulff 1937 b
arvense Scop. 34 Poddubnaja-Arnoldi 1931
helenioides Hill 34 Wulff 1937 b
 [as *heterophyllum* Hill]
palustre Scop. 34 Poddubnaja-Arnoldi 1931
vulgare Shaw 68 Tischler 1927 a
 [as *lanceolatum* Scop.]
dissectum Hill .. Poddubnaja-Arnoldi 1931
 (pratense Druce)
eriphorum Scop. ..
tuberosum All. ..
232. ONOPORDUM $x=17$
Acanthium L. 34 Poddubnaja-Arnoldi 1931
233. SILYBUM $x=?$
Marianum Gaertn. ..
234. SAUSSUREA $x=13$
alpina DC. ..
235. SERRATULA $x=11$
tinctoria L. 22 Maude unpub.
236. CENTAUREA
 $x=10, 11, 12$
Scabiosa L. 20 Roy 1937
 Marsden-Jones
 Turrill 1937 (G, S)
aspera L. 22 Maude unpub.
Cyanus L. 24 Poddubnaja-Arnoldi 1927
Jacea L. 44 Roy 1937
 Wulff 1937 b
nemoralis Jord. 44 Roy 1937
pratensis Thuill. 44
 (nigra Linn. agg.)
angustifolia Gugl. ..
Calcitrapa L. ..
Drucei C. E. Britton ..
jungens Gugl. ..
memphala Jord. ..
obscura Jord. ..
paniculata L. ..
solstitialis L. ..
subjacea Hayk. ..
surrejana C. E. Britton ..
viretorum Jord. ..
237. CICHORIUM $x=?$
Intybus L. c. 18 Makowsky 1929
238. ARNOSERIS $x=9$
minima Schweigg & Koerte 18 Maude unpub.
239. LAPSANA $x=6$
communis L. 12 Marchal 1920
240. PICRIS $x=4, 5$ (apo) 8 Marchal 1920
echinoides L. 10 Bergman 1932
hieracioides L.
241. CREPIS $x=3$
capillaris Wallr. 6 Babcock & Cameron 1934 (G, S)
 Richardson 1935 a, b
nicoensis Balb. 8 Hollingshead & Babcock 1930
 Babcock & Emsweller 1936
setosa Hall f. 8 Hollingshead & Babcock 1930
taraxacifolia Thuill. 8
foetida L. 10
mollis Aschers. 12
paludosa Moench 12
biennis L. 40
242. HIERACIUM (apo) $x=9$ 39-45 Rosenberg 1917, 1926, 1927
 Ostenfeld 1921 (G)
 Gustafsson 1935 (A)
umbellatum (L.) Zahn 18, 27 Rosenberg 1927
alpinum L. 27 Rosenberg 1926
gothicum Fr. 27 Christoff & Popoff 1933
tridentatum Fr. 27 Sakai 1935
aurantiacum NP. 36
pilosella L. 36 Christoff & Popoff 1933

- prenanthoides* Vill. 36 Christoff & Popoff 1933
pulmonarioides Vill. 36 Gentschef 1937 a
- 248 species.
 Sexual species:
 diploid 18
 Apomictic species:
 mostly triploid 27
 some tetraploid (e.g. pilosella) 36
 some pentaploid 45
243. HYPOCHOERIS
 $x=4, 5$
radicata L. 8 Nawaschin (T. 1927 a)
maculata L. 10 Wulff unpub.
glabra L. 10 Negodi 1936 b
244. LEONTODON
 $x=5, 6, 7$ (apo)
taraxacoides Lacaita 10 Wulff 1937 b
 [as *Thrinia hirta* Roth]
autumnale L. 12 Bergman 1935 b (G)
hispidum L. 14
245. TARAXACUM
 $x=8$ (apo)
laevigatum DC. 24 Gustafsson 1935 (A)
obliquum Dahlst. 24 Poddubnaja-Arnoldi & Dianowa 1934
 Gustafsson 1932 a
 (G, S)
vulgare Schrank 24 Gustafsson 1932 a, b
 Okabe 1934
paludosum Schlecht. 32 Poddubnaja-Arnoldi & Dianowa 1934
 Gustafsson 1932 a
- spectabile* Dahlst. 32, 40?
246. LACTUCA $x=4, 5, 9$
virosa L. 18 Babcock, Stebbins & Jenkins 1937
marialis Gaertn. 8, 18
Seriola L. 18
 [as *L. Scariola* L.]
saligna L. 18
alpina Benth. ..
247. SONCHUS $x=8, 9$
oleraceus L. 16 Marchal 1920
 Cooper & Mahony 1935
palustris L. 18 Wulff 1937 b
arvensis L. 64
asper Hill ..
248. TRAGOPOGON $x=7, 9$
pratense L. 12 Winge 1938 (G)
porrifolium L. 12
minus Mill. ..
249. SCORZONERA $x=7$
humilis L. 14 Wulff 1938

CAMPANULACEAE

250. LOBELIA $x=7, 8$
urens L. 14 de Vilmorin & Simonet 1927
 Armand 1912
Dortmanna L. 16
 251. JASIONE $x=6$
montana L. 12 Wulff 1937 b
252. WAHLENBERGIA
 $x=?$
hederacea Reichb. ..
253. PHYTEUMA $x=?$
spicatum L. 36 Armand 1912
orbiculare L. ..
254. CAMPANULA
 $x=8, 10, 17$
persicifolia L. 16 Gairdner & Darlington 1932 (G, S)
Rapunculus L. 20 Marchal 1920
 102 Sugiura 1937
glomerata L. 34 Marchal 1920
latifolia L. 34 de Vilmorin & Simonet 1927
Trachelium L. 34 Marchal 1920
rotundifolia L. 68 Boecher 1936

CAMPANULA (cont.)

<i>rapunculoides</i> L.	102	de Vilmorin & Simonet 1927 Belling (T. 1931)
<i>patula</i> L.
255. <i>LEGOUSIA</i> $x=?$		
<i>hybrida</i> Delarbre

VACCINIACEAE

256. <i>VACCINIUM</i> $x=12$		
<i>Vitis-Idaea</i> L.	24	Hagerup 1928
<i>uliginosum</i> L.	24, 48	Hagerup 1933
<i>Myrtillus</i> L.
257. <i>OXYCOCCUS</i> $x=?$		
<i>quadripetala</i> Gilib.	72	Hagerup 1928

ERICACEAE

258. <i>ARBUTUS</i> $x=?$		
<i>Unedo</i> L.
259. <i>ARCTOSTAPHYLOS</i> $x=?$		
<i>Uva-ursi</i> Spreng.	52	Hagerup 1928
<i>alpina</i> Niedenger
260. <i>ANDROMEDA</i> $x=?$		
<i>Polifolia</i> L.	48	Hagerup 1928
261. <i>CALLUNA</i> $x=8$		
<i>vulgaris</i> Hull	16	Hagerup 1928
262. <i>ERICA</i> $x=12$		
<i>cinerea</i> L.	24	Wanscher 1933 b
<i>Tetralix</i> L.	24	Hagerup 1928
<i>vagans</i> L.	24	Maude unpub.
<i>ciliaris</i> L.
<i>Mackayi</i> Hook.
<i>mediterranea</i> L.
263. <i>AZALEA</i> $x=12$		
<i>procumbens</i> L.	24	Hagerup 1928
[as <i>Loiseleuria procumbens</i> Desv.]		
264. <i>LEDUM</i> $x=13$		
<i>palustre</i> L.
265. <i>PHYLLODOCE</i> $x=12$		
<i>caerulea</i> Bab.	24	Wanscher 1933 b
(<i>Bryanthus caeruleus</i> Dippel)		
266. <i>DABOECIA</i> $x=12$		
<i>cantabrica</i> K. Koch	24	Maude unpub.
267. <i>PYROLA</i> $x=?$		
<i>media</i> Sw.	c. 32	Samuelsson 1913
<i>rotundifolia</i> L.	42	Hagerup 1928
<i>minor</i> L.	46	..
<i>secunda</i> L.
268. <i>MONESSES</i> $x=?$		
<i>uniflora</i> A. Gray	32	Samuelsson 1913
[as <i>Pyrola uniflora</i> L.]		

MONOTROPACEAE

269. <i>MONOTROPA</i> $x=?$		
<i>Hypopitys</i> L.

PLUMBAGINACEAE

270. <i>LIMONIUM</i> $x=?$		
<i>vulgare</i> Mill.	36	Wulff 1937 a
[as <i>Statice Limonium</i> L.]		
<i>bellidifolium</i> Dum.
<i>binervosum</i> C. E. Salmon
<i>humile</i> Mill.
<i>lychnidifolium</i> O. Kuntze
<i>paradoxum</i> Pugsf.
<i>recurvum</i> C. E. Salmon
<i>transwallianum</i> Pugsf.
271. <i>ARMERIA</i> $x=9$		
<i>maritima</i> Willd.	14	Fernandes 1931 a
	18	Griesinger 1937
	18	Phillips 1938
<i>plantaginea</i> Willd.
<i>pubescens</i> Link

PRIMULACEAE

272. <i>HOTTONIA</i> $x=?$		
<i>palustris</i> L.	20	Wulff 1938
273. <i>PRIMULA</i> $x=9, 11$		
<i>farinosa</i> L.	18	Wright Smith 1933 (S)
<i>elatior</i> Jacq.	22	Bruun 1932 a, b
		Bruun 1932 b
<i>veris</i> L.	22	Chittenden 1928 (G)
<i>vulgaris</i> Huds.	22	Bruun 1932 b
<i>scotica</i> Hook.	72	..
274. <i>CYCLAMEN</i> $x=?$		
<i>hederifolium</i> Ait.	28-32	Heitz 1926
[as <i>C. europaeum</i> L.]		
275. <i>LYSIMACHIA</i> $x=?$		
<i>nemorum</i> L.	18	Wulff 1938
<i>vulgaris</i> L.	28	Lewitzky 1934
<i>Nummularia</i> L.	36	Wulff 1938
<i>thyrsiflora</i> L.	c. 40	Dahlgren 1916
<i>ciliata</i> L.
<i>punctata</i> L.
<i>terrestris</i> Britton
276. <i>TRIENTALIS</i> $x=?$		
<i>europaea</i> L.	c. 160	Wulff 1937 b
277. <i>GLAUX</i> $x=?$		
<i>maritima</i> L.	30	Wulff 1937 a
278. <i>ANAGALLIS</i> $x=10, 11$		
<i>tenella</i> Murr.	22	Maude unpub.
<i>arvensis</i> Mill.	40	Wulff 1937 b
		Marsden-Jones 1935 (G)
<i>foemina</i> Mill.	..	Nilsson 1938 (G)
		Marsden-Jones 1935 (G)
279. <i>CENTUNCULUS</i> $x=?$		
<i>minimus</i> L.
280. <i>SAMOLUS</i> $x=?$		
<i>Valerandi</i> L.	c. 24	Wulff 1937 a

OLEACEAE

281. <i>FRAXINUS</i> $x=23$		
<i>excelsior</i> L.	46	Sax & Abbe 1932
282. <i>LIGUSTRUM</i> $x=23$		
<i>vulgare</i> L.	46	Sax & Abbe 1932

APOCYNACEAE

283. <i>VINCA</i> $x=8$		
<i>major</i> L.	16	Schürhoff & Müller 1937
<i>minor</i> L.	32	..
	46?	Finn 1928

GENTIANACEAE

284. <i>MICROCALA</i> $x=?$		
<i>filiformis</i> Hoffmgg. & Link
285. <i>BLACKSTONIA</i> $x=11$		
<i>perfoliata</i> Huds.	44	Maude unpub.
286. <i>CENTAURIUM</i> (<i>ERYTHRAEA</i>) $x=?$		
<i>pulchellum</i> Druce	c. 34	Warburg unpub.
	c. 38	Wulff 1937 a
<i>littorale</i> Gilmour	c. 38	..
	38, c. 56	Warburg unpub.
<i>capitatum</i> Britton & Rendle
<i>latifolium</i> Druce
<i>scilloides</i> var. <i>portense</i> Druce
(<i>portensis</i> Hoffmgg. & Link)
<i>temiflorum</i> Fritsch
<i>umbellatum</i> Gilib.
287. <i>CICENDIA</i> $x=?$		
<i>pusilla</i> Griseb.
288. <i>GENTIANA</i> $x=?$		
<i>Amarella</i> L.
<i>anglica</i> Pugsf.

GENTIANA (cont.)

<i>baltica</i> Murb.
<i>campestris</i> L.
<i>germanica</i> Willd.
<i>nivalis</i> L.
<i>Pneumonanthe</i> L.
<i>uliginosa</i> Willd.
<i>verna</i> L.
289. <i>MENYANTHES</i> $x=?$			
<i>trifoliata</i> L.	54?	Matsuura & Suto	1935
290. <i>NYMPHOIDES</i> $x=?$			
<i>peltatum</i> Britton & Rendle

POLEMONIACEAE

291. <i>POLEMONIUM</i> $x=9$			
<i>coeruleum</i> L.	18	Clausen 1931	Heitz 1932

BORAGINACEAE

292. <i>CYNOGLOSSUM</i> $x=?$			
<i>officinale</i> L.	24?	Strey 1931	
<i>montanum</i> L.
293. <i>ASPERUGO</i> $x=?$			
<i>procumbens</i> L.
294. <i>SYMPHYTUM</i> $x=6$			
<i>officinale</i> L.	c. 36	Strey 1931	
<i>peregrinum</i> Ledeb.	36	Maude unpub.	
<i>tuberosum</i> L.	72	Strey 1931	
<i>orientale</i> L.
295. <i>BORAGO</i> $x=8$			
<i>officinalis</i> L.	16	Strey 1931	
296. <i>ANCHUSA</i> $x=6, 8, 11$			
<i>officinalis</i> L.	16	Lewitzky 1934	
<i>sempervirens</i> L.	22	S. G. Smith, 1932	
	16	Sugiura 1937b	
	22	Maude unpub.	
297. <i>LYCOPSIS</i> $x=?$			
<i>arvensis</i> L.	54	Svensson 1925	
298. <i>PULMONARIA</i> $x=7$			
<i>longifolia</i> Boreau	14	Tarnavski 1935	
<i>officinalis</i> L.	14	Strey 1931	
299. <i>MERTENSIA</i> $x=?$			
<i>maritima</i> Gray
300. <i>MYOSOTIS</i> $x=8, 12$			
<i>silvatica</i> Hoffm.	18, 32	Geitler 1936 (S)	
<i>alpestris</i> Schmidt	24, 48, 72?	"	
<i>collina</i> Hoffm.	48	"	
<i>arvensis</i> Hill	54?	"	
<i>versicolor</i> Sm.	c. 60	Winge 1917	
<i>palustris</i> Hill	64	Strey 1931	
(<i>scorpioides</i> L.)			
<i>caespitosa</i> Schultz	80	"	
<i>brevifolia</i> C. E. Salmon
<i>repens</i> G. & D. Don
<i>sicula</i> Gurs.
301. <i>LITHOSPERMUM</i> $x=6, 7$			
<i>officinale</i> L.	28?	Strey 1931	
<i>arvense</i> L.
<i>purpureo-coeruleum</i> L.
302. <i>ECHIMUM</i> $x=8$			
<i>plantagineum</i> L.	16	Sugiura 1931	
<i>vulgare</i> L.	32	Strey 1931	

CONVOLVULACEAE

303. <i>CALYSTEGIA</i> $x=11$			
<i>sepium</i> Br.	22	Kano 1929	
	24	Wolcott 1937	
<i>Soldanella</i> Br.	22	Kano 1929	
304. <i>CONVOLVULUS</i> $x=?$			
<i>arvensis</i> L.	50	Wolcott 1937	
305. <i>CUSCUTA</i> $x=7$			
<i>Epithymum</i> Murr.	14	Finn & Safijovska	1934
<i>europaea</i> L.	14	"	
<i>Epilinum</i> Weihe	42	"	
<i>Trifolii</i> Bab.

SOLANACEAE

306. <i>SOLANUM</i> $x=12$			
<i>Dulcamara</i> L.	24	de Vilmorin & Simonet 1928	
		Jørgensen 1928 (G)	
<i>nigrum</i> L.	24, 48, 72	Bhaduri 1933	
	72	Tokunaga 1934	
		Winkler 1934	
307. <i>LYCIUM</i> $x=12$			
<i>chinense</i> Mill.	24	Sugiura 1931	
308. <i>ATROPA</i> $x=12$			
<i>Belladonna</i> L.	72	de Vilmorin & Simonet 1928	
309. <i>DATURA</i> $x=12$			
<i>Stramonium</i> L.	24	Blakeslee 1934 (S, G)	
310. <i>HYOSCYAMUS</i> $x=17$			
<i>niger</i> L.	34	de Vilmorin & Simonet 1928	
		Griesinger 1937	

SCROPHULARIACEAE

311. <i>VERBASCUM</i> $x=8$			
<i>Blattaria</i> L.	30	Håkansson 1926	
<i>nigrum</i> L.	30	"	
<i>Lychnitis</i> L.	32	"	
<i>pulverulentum</i> Vill.	32	Perino 1915	
<i>thapsiforme</i> Schrad.	32	Håkansson 1926	
<i>virgatum</i> Stokes	32	"	
<i>Thapsus</i> L.	(34) 36	"	
		Lawrence 1931	
312. <i>LINARIA</i> $x=6, 7$			
<i>purpurea</i> Mill.	12	Bruun 1932 (G)	
<i>repens</i> Mill.	12	Heitz 1927a	
<i>supina</i> Desf.	12	Tjebbes 1928	
<i>vulgaris</i> Mill.	12	Heitz 1927a	
<i>Cymbalaria</i> Mill.	14	East 1933	
<i>Elatine</i> Mill.	..	"	
<i>minor</i> Mill.
<i>Pelissieriana</i> Mill.
<i>spuria</i> Mill.
313. <i>ANTIRRHINUM</i> $x=8$			
<i>majus</i> L.	16	Baur 1932 (S, G)	
		Schick 1934	
		Propach 1934	
	17	Noethling & Stubbe	
		1934	
<i>Orontium</i> L.	18	Stubbe 1934a, b, c	
	16	Heitz 1927a, b	
314. <i>SCROPHULARIA</i> $x=10?$			
<i>vernalis</i> L.	40	Håkansson 1926	
<i>aquatica</i> L.	80	Maude unpub.	
<i>alata</i> Gilib.
<i>nodosa</i> L.
<i>Scorodonia</i> L.
315. <i>MIMULUS</i> $x=8$			
<i>guttatus</i> DC.	48	Maude unpub.	
(<i>Langsdorffii</i> Donn)			
316. <i>LIMOSSELLA</i> $x=?$			
<i>aquatica</i> L.	36	Svensson 1928	
<i>tenuifolia</i> Wolf.
317. <i>SIBTHORPIA</i> $x=?$			
<i>europaea</i> L.
318. <i>DIGITALIS</i> $x=7$			
<i>purpurea</i> L.	56	Buxton & Dark 1934 (G)	
319. <i>VERONICA</i> $x=7, 8, 9$			
<i>polita</i> Fr.	14	Beatus 1936	
<i>repens</i> DC.	14	Lehmann 1937	
<i>serpyllifolia</i> L.	14	"	
<i>triphyllus</i> L.	14	"	
<i>agrestis</i> L.	28	Wulff 1937b	
<i>persica</i> Poir.	28	Beatus 1934	
[<i>as Tournefortii</i> Gmel.]			
<i>arvensis</i> L.	16	Lehmann 1937	
<i>fruticans</i> Jacq.	16	Simonet 1934a	
<i>verna</i> L.	16	Lehmann 1937	
<i>Chamaedrys</i> L.	32	Simonet 1934a	
<i>alpina</i> L.	18	Maude unpub.	
<i>montana</i> L.	18	"	
<i>Beccabunga</i> L.	18	Schlenker 1936	
<i>officinalis</i> L.	34-38	Simonet 1934a	
<i>Anagallis-aquatica</i> L.	36	Schlenker 1936	

VERONICA (cont.)

<i>spicata</i>	34, 68	Grise 1933
	64-68	Simonet 1934 d
<i>peregrina</i> L.	52	Lehmann 1937
<i>hederifolia</i> L.	56	"
<i>acutifolia</i> L.
<i>aquatica</i> Beccarel
<i>humifusa</i> Dickson
<i>hybrida</i> L.
<i>praecox</i> All.
<i>scutellata</i> L.
320. EUPHRASIA $\alpha=11$		
<i>confusa</i> Pugsley	44	v. Witsch 1932
(<i>minima</i> Auctt. angl.)		
<i>salisburgensis</i> Funck	44	"
17 other species.		
321. BARTSIA $\alpha=?$		
<i>Odontites</i> Huds.	20	Tischler 1935
		Fagerlind 1937 a
<i>alpina</i> L.	24	v. Witsch 1932
<i>viscosa</i> L.
322. PEDICULARIS $\alpha=8$		
<i>palustris</i> L.	16	v. Witsch 1932
<i>sylvatica</i> L.
323. RHINANTHUS $\alpha=7$		
<i>major</i> Ehrh.	14	Wulff 1937 b
[as <i>Alectorolophus major</i>	14 + 8 ff.	Fagerlind 1937 a
Reichb.]		
<i>minor</i> Ehrh.	14	v. Witsch 1932
[as <i>Alectorolophus minor</i>		
Dum.]		
<i>borealis</i> Druce
<i>Drummond-Hayii</i> Druce
<i>groenlandicus</i> Chab.
<i>monticola</i> Druce
<i>Perrieri</i> Chab.
<i>stenophyllus</i> Schür.
324. MELAMPYRUM $\alpha=9$		
<i>arvense</i> L.	18	v. Witsch 1932
<i>pratense</i> L.	18	"
<i>sylvaticum</i> L.	18	"
<i>cristatum</i> L.

OROBANCHACEAE

325. OROBANCHE $\alpha=?$		
<i>minor</i> Sm.	38	K. M. Carter, (T. 1931)
<i>amethystea</i> Thuill.
<i>arenaria</i> Borkh.
<i>caryophyllacea</i> Sm.
<i>elator</i> Sutton
<i>Hederae</i> Duby
<i>major</i> L.
<i>Picridis</i> F. Schultz
<i>purpurea</i> Jacq.
<i>reticulata</i> Wallr. var. <i>procera</i>
Koch		
<i>Ritro</i> Gren. & Godr.
<i>rubra</i> Sm.
326. LATHRAEA $\alpha=?$		
<i>Squamaria</i> L.	42	Gates & Latter 1927

LENTIBULARIACEAE

327. UTRICULARIA $\alpha=?$		
<i>Bremii</i> Heer
<i>intermedia</i> Hayne
<i>major</i> Schmidel
<i>minor</i> L.
<i>ochroleuca</i> Hartm.
<i>vulgaris</i> L.
328. PINGUICULA $\alpha=?$		
<i>vulgaris</i> L.	50	Rosenberg 1909 b
<i>alpina</i> L.
<i>grandiflora</i> Lam.
<i>lusitanica</i> L.

VERBENACEAE

329. VERBENA $\alpha=5, 7$		
<i>officinalis</i> L.	14	Noack 1937

LABIATAE

330. MENTHA $\alpha=9, 10, 12$		
<i>Pulegium</i> L.	20, 40	Ruttie 1931
<i>longifolia</i> Huds.	24	"
	18	Heimans 1938
<i>rotundifolia</i> Huds.	24	Ruttie 1931
	18	Heimans 1938
<i>viridis</i> L.	36	Schürhoff 1929
<i>piperita</i> L.	66, 68, 70	Ruttie 1931
<i>arvensis</i> L.	72	"
<i>aquatica</i> L.	96 (94, 98)	"
<i>alopecuroides</i> Hull
<i>cardiaca</i> Baker
<i>citrata</i> Ehrh.
<i>gentilis</i> L.
<i>gracilis</i> Sm.
<i>rubra</i> Sm.
331. LYCOPUS $\alpha=11$		
<i>europaeus</i> L.	22	Ruttie 1932
332. ORIGANUM $\alpha=?$		
<i>vulgare</i> L.
333. THYMUS $\alpha=?$		
<i>Serpyllum</i> L.	c. 20	Némec 1925
<i>ovatus</i> Mill.
334. CLINOPODIUM $\alpha=?$		
<i>vulgare</i> L.
335. CALAMINTHA $\alpha=?$		
<i>Acinos</i> Clairv.
<i>ascendens</i> Jord.
<i>baetica</i> Boiss.
<i>Nepeta</i> Savi
<i>sylvatica</i> Bromf.
336. MELISSA $\alpha=?$		
<i>officinalis</i> L.
337. SALVIA		
$\alpha=6, 7, 8, 9, 10, 11$		
<i>verticillata</i> L.	16	Hrubý 1934 (G)
<i>pratensis</i> L.	18	"
<i>horminoides</i> Pourr.	64	Yakovleva 1933
<i>Verbenaca</i> L.	64	"
	54	Benoist 1937
338. NEPETA $\alpha=8$		
<i>Cataria</i> L.	32	Bushnell 1936
		Sugiura 1937 b
<i>hederacea</i> Trev.
339. SCUTELLARIA $\alpha=8$		
<i>galericalata</i> L.	c. 32	Scheel 1931
<i>minor</i> Huds.
340. PRUNELLA $\alpha=8$		
<i>laciniata</i> L.	32	Hrubý 1932
<i>vulgaris</i> L.	32	"
341. MELITIS $\alpha=?$		
<i>Melissophyllum</i> L.
342. MARRUBIUM $\alpha=?$		
<i>vulgare</i> L.
343. STACHYS $\alpha=8$		
<i>officinalis</i> Trev.	16	Lewitzky (T. 1936)
[as <i>Betonica officinalis</i> L.]		Turesson 1938
<i>palustris</i> L.	c. 64	Wulff 1938
<i>alpina</i> L.
<i>annua</i> L.
<i>arvensis</i> L.
<i>germanica</i> L.
<i>sylvatica</i> L.
344. GALEOPSIS $\alpha=8$		
<i>angustifolia</i> Ehrh.	16	Müntzing 1930 a
		(G)
<i>dubia</i> Leers	16	Müntzing 1930 a
[as <i>ochroleuca</i> Lam.]		
<i>intermedia</i> Vill.	16	"
(<i>Ladanum</i> L.)		
<i>speciosa</i> Mill.	16	Müntzing 1930 a, b
		1931 a 1938 (G)
<i>Tetrahit</i> L.	32, 33	Müntzing 1932 a, b
		1938 (G)
345. LEONURUS $\alpha=?$		
<i>Cardiaca</i> L.
346. LAMNUM $\alpha=9$		
<i>album</i> L.	18	Jorgensen 1927 (G)
<i>amplexicaule</i> L.	18	"
<i>maculatum</i> L.	18	"
<i>purpureum</i> L.	18	"

LAMIUM (cont.)

<i>Galeobdolon</i> Crantz	36	Jorgensen 1927 (G)
<i>mollucellifolium</i> Fr.	36	"
[as <i>intermedium</i> Fr.]		
<i>hybridum</i> Vill.
347. <i>BALLOTA</i> $x=?$		
<i>nigra</i> L.
<i>ruderalis</i> Sw.
348. <i>TEUCRIUM</i> $x=5, 8$		
<i>Botrys</i> L.	10	Junell 1934
<i>Chamaedrys</i> Schreb.
<i>Scordium</i> L.
<i>Scorodonia</i> L.
349. <i>AJUGA</i> $x=8$		
<i>reptans</i> L.	32	Maude unpub.
<i>Chamaedrys</i> Schreb.
<i>genevensis</i> L.
<i>pyramidalis</i> L.

PLANTAGINACEAE

350. <i>PLANTAGO</i> $x=5, 6$		Gregor 1937 (G, S)
<i>Coronopus</i> L.	10	MacCullagh 1934
<i>Lagopus</i> L.	12	Wulff 1937a
<i>lanceolata</i> L.	12	MacCullagh 1934
		MacCullagh (T. 1936)
<i>major</i> L.	12	MacCullagh 1934
<i>maritima</i> L.	12	"
		Wulff 1937a
<i>Psyllium</i> L.	12	MacCullagh 1934
<i>ramosa</i> Asch.	12	"
<i>Cynops</i> L.	24	"
<i>media</i> L.	24	"
351. <i>LITTORELLA</i> $x=?$		
<i>uniflora</i> Asch.

ILLECEBRACEAE

352. <i>ILLECEBRUM</i> $x=?$		
<i>verticillatum</i> L.
353. <i>HERNIARIA</i> $x=?$		
<i>ciliata</i> Bab.
<i>glabra</i> L.
<i>hirsuta</i> L.
354. <i>CORRIGIOLA</i> $x=9$		
<i>littoralis</i> L.	18	Blackburn (T. 1936)
355. <i>SCLERANTHUS</i>		
$x=11$		
<i>annuus</i> L.	22	Rohweder unpub.
<i>perennis</i> L.	44	Blackburn (T. 1936)

AMARANTHACEAE

356. <i>AMARANTHUS</i> $x=?$		
<i>Blitum</i> L.	35	Tagaki 1933
<i>retroflexus</i> L.

CHENOPODIACEAE

357. <i>CHENOPODIUM</i> $x=9$		
<i>capitatum</i> Asch.	18	Kjellmark 1934
<i>ficifolium</i> Sm.	18	"
<i>glaucum</i> L.	18	Wulff 1936
<i>leptophyllum</i> Moq.	18	Kjellmark 1934
<i>murale</i> L.	18	Winge 1917
<i>polyspermum</i> L.	18	Kjellmark 1934
<i>Vulvaria</i> L.	18	Winge 1917
<i>album</i> L.	18	Maude unpub.
	36	G. O. Cooper, 1935
<i>Bonus-Henricus</i> L.	54	Kjellmark 1934
<i>hybridum</i> L.	36	Wulff 1936
<i>opulifolium</i> Schrad.	36	G. O. Cooper, 1935
<i>rubrum</i> L.	36	"
<i>Berlandieri</i> Moq.	36	Kjellmark 1934
<i>botryoides</i> Sm.
<i>hircinum</i> Schrad.
<i>uribicum</i> L.
358. <i>BETA</i> $x=9$		
<i>maritima</i> L.	18	Claus 1933

359. *ATRIPLEX* $x=9$

<i>hastata</i> L.	18	Winge 1917
<i>hortensis</i> L.	18	Kjellmark 1934
<i>littoralis</i> L.	18	O. G. Wulff 1937a
<i>patula</i> L.	18	Kjellmark 1934
		Cooper 1935
		Wulff 1936
<i>pedunculata</i> L.	18, 36	
[as <i>Obione pedunculata</i> Moq.]		
<i>portulacoides</i> L.	36	"
[as <i>Obione portulacoides</i> Moq.]		
<i>Babingtonii</i> Woods
<i>glabriuscula</i> Edmondston
<i>Sabulosa</i> Roug.
(<i>maritima</i> E. Hallier)

360. *SALICORNIA* $x=9$

[as <i>S. perennis</i>]	18	Maude unpub.
[as <i>S. annua</i>]	36	"
<i>appressa</i> Dum.
<i>disarticulata</i> Moss
<i>dolichostachya</i> Moss
<i>gracillima</i> Moss
<i>lignosa</i> Woods
<i>pusilla</i> Woods
<i>radicans</i> Sm.
<i>ramosissima</i> Woods
<i>stricta</i> Dum.

361. *SUAEDA* $x=9$

<i>maritima</i> Dum.	36	Wulff 1937a
		Maude unpub.
<i>fruticosa</i> Forssk.	36	Joshi 1935

362. *SALSOLA* $x=9$

<i>Kali</i> L.	36	Wulff 1937a
<i>Tragus</i> L.	36	Wulff 1936

POLYGONACEAE

363. *POLYGONUM*

$x=10, 11$		
<i>Convolvulus</i> L.	20	Jaretsky 1928 b
<i>dumetorum</i> L.	20	"
<i>Hydropiper</i> L.	20	"
<i>maritimum</i> L.	20	"
<i>lappathifolium</i> L.	22	"
<i>aviculare</i> * L.	40	"
<i>Bistorta</i> L.	44	"
<i>Persicaria</i> L.	44	"
<i>amphibium</i> L.	c. 66	"
<i>viviparum</i> L.	c. 110	"
<i>calcatum</i> Lindman
<i>laxiflorum</i> Weihe
<i>minus</i> Huds.
<i>nodosum</i> Pers.
<i>oxyspermum</i> Mey. & Bge.
<i>Raii</i> Bab.
<i>sagittatum</i> L.

364. *FAGOPYRUM* $x=8$

<i>sagittatum</i> Gilib.	16	Quisenberry 1927
[as <i>esculentum</i> Moench]		Jaretsky 1927 b

365. *OXYRIA* $x=7$

<i>digyna</i> Hill	14	Edman 1929
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366. *RUMEX* $x=7, 8, 9, 10$

<i>Acetosa</i> L.	14 ♀ (XX) 15 ♂ (XY)	Yamamoto 1934 a, b (G)
		Jensen 1937 a, b
Same in triploid and hexaploid varieties.		
<i>arifolius</i> All.	14, 16	Jaretsky 1927
<i>Acetosella</i> L.	42	Ono 1930
<i>alpinus</i> L.	20	Jaretsky 1928 b
<i>glomeratus</i> Schreb.	20	"
[as <i>conglomeratus</i> Murr.]	18	Sugiura 1936
<i>pulcher</i> L.	20	Shimamura 1929
<i>sanguineus</i> L.	20	Jaretsky 1928 b
<i>scutatus</i> L.	20	"
<i>limosus</i> Thuill.	40	Fikry 1930
<i>maritimus</i> L.	40	Jaretsky 1928 b
<i>obtusifolius</i> L.	40	"
<i>crispus</i> L.	60	Jensen, 1937 b
<i>longifolius</i> DC.	80, 60	Kihara & Ono 1926
[as <i>domesticus</i> Hartm.]		
<i>Hydrolapathum</i> Huds.	200	"
<i>condyloides</i> Bieb.

* Jaretsky gives the chromosome number 40 for *P. aviculare* L. The London Catalogue divides this species into *P. heterophyllum* Lindman and *P. aequale* Lindman.

RUMEX (cont.)		
<i>cuneifolius</i> Campd.
<i>elongatus</i> Guss.
<i>maximus</i> Schreb.
<i>obovatus</i> Danser
<i>rupestris</i> Le Gall

ARISTOLOCHIACEAE

367. ASARUM $x=?$		
<i>europaeum</i> L.	c. 24	Täckholm & Söderberg 1918
368. ARISTOLOCHIA		
<i>Clematidis</i> L.	$x=7$ 14	Samuelsson 1914

THYMELEACEAE

369. DAPHNE $x=9$		
<i>Mexereum</i> L.	18	Maude unpub.
<i>Laureola</i> L.

ELEAGNACEAE

370. HIPPOPHAE $x=12$		
<i>Rhamnoides</i> L.	24	Sobolewska 1928 Hyrfe (T. 1936)

LORANTHACEAE

371. VISCUM $x=10$		
<i>album</i> L.		Steindl 1935

SANTALACEAE

372. THESIUM $x=?$		
<i>humifusum</i> DC.

EUPHORBIACEAE

373. EUPHORBIA		Schnarf 1929 (A)
$x=6, 7, 8, 9, 10$ (apo)		
<i>Peplus</i> L.	16	Wulff 1937b
<i>amygdaloides</i> L.	18	Harrison 1930
<i>dulcis</i> L.	28	Carano 1926
<i>platyphyllus</i> L.	36	Harrison 1930
<i>Helioscopia</i> L.	42	"
<i>coralloides</i> L.
<i>Cyparissias</i> L.
<i>Esula</i> L.
<i>exigua</i> L.
<i>hiberna</i> L.
<i>Lathyrus</i> L.
<i>Paralias</i> L.
<i>Peplus</i> L.
<i>pilosa</i> L.
<i>porilandica</i> L.
<i>stricta</i> L.
<i>virgata</i> Waldst. & Kit.
374. BUXUS $x=7$		
<i>sempervirens</i> L.	28	Simonet & Miedzyrzecki 1932
375. MERCURIALIS $x=8$		
<i>annua</i> L.	16	Yampolsky 1933
<i>perennis</i> L.	> 64	Meurman 1924

URTICACEAE

376. ULMUS $x=14$		
<i>procera</i> Salisb.	28	Sax 1933
[as <i>campestris</i> L.]		Leliveld 1933
<i>glabra</i> Huds.	28	"
[as <i>montana</i> Stokes]		
<i>nitens</i> Moench	28	Maude unpub.
<i>minor</i> Mill.
<i>stricta</i> Lindley
377. HUMULUS $x=8, 10$		
<i>Lupulus</i> L.	20 (♂ XY)	Sinoto 1929a,b
378. URTICA $x=12, 13$		
<i>urens</i> L.	24	Fothergill 1936
<i>pilulifera</i> L.	26	Krause 1931
		Fothergill 1936
<i>dioica</i> L.	48	Heitz 1926
	52	Fothergill 1936

379. PARIETARIA $x=7$		
<i>ramiflora</i> Moench	14	Krause 1930
[as <i>officinalis</i> L.]		

MYRICACEAE

380. MYRICA $x=8$		
<i>Gale</i> L.

CUPULIFERAE

381. BETULA $x=7$		
<i>alba</i> L.	28	Woodworth 1931
[as <i>verrucosa</i> Ehrh.]		
<i>nana</i> L.	28	Jaretsky 1930
<i>pubescens</i> Ehrh.	56	Woodworth 1931
382. ALNUS $x=7$		
<i>incana</i> DC.	28	Woodworth 1931
<i>glutinosa</i> Gaertn.	28	Jaretsky 1930
(<i>rotundifolia</i> Mill.)	56	Woodworth 1931
383. CARPINUS $x=8$		
<i>Betulus</i> L.	16	Woodworth 1931
384. CORYLUS $x=7$		
<i>Avellana</i> L.	28	Woodworth 1920
385. QUERCUS $x=12$		
<i>cerris</i> L.	24	Natividade 1937
<i>flex</i> L.	24	Jaretsky 1930
<i>Robur</i> L.	24	Vignoli 1933
<i>petraea</i> Lieblein	24	Jaretsky 1930
[as <i>sessiliflora</i> Salisb.]		Vignoli 1933
386. CASTANEA $x=12$		
<i>sativa</i> Mill.	24	Jaretsky 1930
387. FAGUS $x=12$		
<i>sylvatica</i> L.	24	Jaretsky 1930

SALICACEAE

388. SALIX $x=19$ (apo)		
<i>caprea</i> L.	38	Schnarf 1929 (A)
		Håkansson 1929a
		Heribert-Nilsson
<i>daphnoides</i> Villars	38	1930 (G)
		Blackburn & Harri-
		son 1924
<i>myrsinites</i> L.	38	"
<i>purpurea</i> L.	38	"
<i>repens</i> L.	38	"
<i>triandra</i> L.	38	"
<i>viminialis</i> L.	38	Håkansson 1929a
<i>aurita</i> L.	38	"
<i>alba</i> L.	76	Harrison 1926
	76	Blackburn & Harri-
		son 1924
<i>cinerea</i> L.	76	Harrison 1926
<i>fragilis</i> L.	76	Blackburn & Harri-
		son 1924
<i>pentandra</i> L.	76	
<i>Andersoniana</i> Sm.	114	Harrison 1926
		Sinoto 1928
<i>phylicifolia</i> L.	114	Håkansson 1933b
<i>arbuscula</i> L.
<i>herbacea</i> L.
<i>lanata</i> L.
<i>lappinum</i> L.
<i>pruinosa</i> Wendl.
<i>reticulata</i> L.
389. POPULUS $x=19$		
<i>alba</i> L.	38	v. Wettstein 1933
<i>nigra</i> L.	38	Blackburn & Harri-
		son 1924
<i>serotina</i> Hartig	38	Blackburn 1929
<i>tremula</i> L.	38	v. Wettstein 1933
<i>canescens</i> Sm.	38, 57	Peto 1938

EMPETRACEAE

390. EMPETRUM $x=13$		
<i>nigrum</i> L.	26	Wanscher 1933b

CERATOPHYLLACEAE

391. CERATOPHYLLUM		
$x=12?$		
<i>demersum</i> L.	c. 24	Langlet & Söder-
		berg 1927
<i>submersum</i> L.	72	Jedrychowska &
		Scoczynska 1934

HYDROCHARIDEAE

392. ELODEA $x=12$
canadensis Michx. 24? Wylie 1904
 48 Santos 1924
393. HYDRILLA $x=8$
verticillata Casp. 16, 24 Sinoto 1929b
394. HYDROCHARIS $x=?$
Morsus-ranae L. 28 Tuschnjakowa
 1929
 Hoare unpub.
395. STRATIOTES $x=?$
Aloides L. 24 Schürhoff 1926

MONOCOTYLEDONES ORCHIDACEAE

396. MALAXIS $x=?$
paludosa Sw.
397. LIPARIS $x=?$
Loeselii Rich.
398. CORALLORHIZA $x=?$
trifida Châtel
399. NEOTTIA $x=9$
Nidus-avis Rich. 36 Modilewski 1936
400. LISTERA $x=?$
cordata Br. 42 Blackburn 1934
ovata Br. 18, 34, 35 Richardson 1933
 34 MacMahon 1936
401. SPIRANTHES $x=?$
aestivalis Rich.
Romanzoffiana Cham.
spiralis Koch
402. GOODYERA $x=10$
repens Br. 30 Richardson 1935c
403. EPIPOGUM $x=?$
aphyllum Sw.
404. CEPHALANTHERA $x=?$
ensifolia Rich.
grandiflora Gray
rubra Rich.
405. EPIPACTIS $x=12$
palustris Sw. 24 Friemann 1910
atropurpurea Raf.
latifolia Sw.
leptochila Godfery
purpurata Sm.
406. ANACAMPTIS $x=10$
pyramidalis Rich. 40 Fuchs & Ziegen-
 speck 1923
407. HIMANTOGLOSSUM $x=12$
hircinum Sprgl. 24 Heusser 1915
 [as *Loroglossum hircinum*
 Rich.]
408. ORCHIS $x=10$
morio L. 36 Hagerup 1938
latifolia L. 40, 80 Richardson unpub.
 80 Hagerup 1938
 [as *incarnata* L.] 40
 [as var. *dunensis* Druce] 40 Richardson unpub.
maculata L. 40, 41 Vermeulen 1938
 (Fuchsii Dr.) 40 Richardson unpub.
 var. O'Kelly Dr. 80 Hagerup 1938
mascula L. 42
militaris L. 42
purpurea Huds. 42
ustulata L. 42
elodes Gris. 80, 79 Richardson unpub.
 [as *O. maculata* var. *erice-*
torum Linton] 80 Hagerup 1938
praetermissa Druce 80 Vermeulen 1938
 var. *pulchella* Dr. 80, 82 Richardson unpub.
purpurella Stephenson 80 Vermeulen 1938
- laxiflora* Lam.
simia Lam.
409. ACERAS $x=?$
anthropophora Br.
410. OPHRYS $x=?$
muscifera Huds. 22-24 Senianinova 1925
 [as *O. myodes* Jacq.]

- apifera* Huds.
fuciflora Reichb.
sphegodes Mill.
411. HERMINIUM $x=?$
monorchis Br. 24-26 Baranow 1925
412. GYMNADENIA $x=10$
conopsea Br. 16? Chodat 1924
 20 Fuchs & Ziegen-
 speck 1923
odoratissima Rich. 40 Richardson 1935c
 20 Fuchs & Ziegen-
 speck 1923
413. NEOTINEA $x=?$
intacta Reichb. f.
414. LEUCHORCHIS $x=?$
albida Mey.
415. COELOGLOSSUM $x=10$
viride Hartm. 40 Richardson 1935c
416. PATLANTHERA $x=?$
bifolia Reichb. f. 42 Richardson 1935c
chlorantha Reichb. 42 "
417. CYPRIPEDIUM $x=9, 10, 11, 13$, etc.
calceolus L. 22 Francini 1931

IRIDACEAE

418. IRIS $x=7, 8, 9, 10, 12$, etc.
tuberosa L. 20 Simonet 1928
 [as *Hemodactylus tuberosus*
 Mill.]
Pseudacorus L. 24 Longley 1928
 34 Simonet 1928
foetidissima L. 30 Heppell unpub.
spuria L. 40 Simonet 1932a
 44 "
419. CROCUS $x=3, 4, 5, 6, 7$, etc.
officinalis Huds. 8, 18, 19 Mather 1932
 [as *vernus* All.] 16 Kihara et al. 1931
 32 Karasawa 1932
nudiflorus Sm. c. 46 Mather 1932
420. ROMULEA $x=?$
parviflora Bubani c. 60 Fabergé unpub.
421. SISYRINCHIUM $x=?$
californicum Ait. 34 Maude unpub.
angustifolium Mill.
422. GLADIOLUS $x=15$
illyricus Koch

AMARYLLIDACEAE

423. NARCISSUS $x=7$
Pseudo-Narcissus L. 21, 22 Nagao 1933
 14, 28 Fernandes 1934
incomparabilis Mill. 14, 21 Nagao 1933
 14 Fernandes 1934
obvallaris Salisb. 14 Philp 1934
odorus L. 14 Fernandes 1934
poeticus L. 14, 21, 28 de Mol 1929
 Nagao 1929, 1933
biflorus Curt. 24 Stomps 1919
major Curt.
424. GALANTHUS $x=12$
nivalis L. 24 Perry 1932
425. LEUCOJUM $x=7, 11$
aestivum L. 22 La Cour 1931
vernum L. 22 Sato 1937

DIOSCOREACEAE

426. TAMUS $x=?$
communis L. 48 Meurman 1925

LILIACEAE

427. RUSCUS $x=?$
aculeatus L. 36 Fernandes 1931a, b
 40 Maude unpub.

428. ASPARAGUS $x=10$
altilis Asch. 20 Flory 1932
 [as *officinalis* L.]
 (*maritimus* Mill.)
429. POLYGONATUM
 $x=7, 9, 10$
multiflorum All. 18 von Berg 1933
 18, 30 Dark unpub.
officinale All. 29 Dark unpub.
 var. *japonicum* Maxim. 20 Hasegawa 1933
 $n=13, 14, 15$ Maude unpub.
verticillatum All. 28 von Berg 1933
 30, 84 Dark unpub.
430. MAIANTHEMUM
 $x=?$
bifolium Schmidt (30-)38 Stenar 1935
 38? Dark unpub.
 c. 42 Maude unpub.
431. CONVALLARIA $x=?$
majalis L. 38 von Berg 1933
 Matsuura & Sutô 1935
432. SIMETHIS $x=?$
planifolia Gren. & Godr.
433. ALLIUM
 $x=7, 8, 9$ (apo)
ursinum L. 14 Levan 1935
Schoenoprasum L. 16, 32 Levan 1936 (G, S)
Scorodoprasum L. 16 Levan 1935
sibiricum L. 16 Maude unpub.
sphaerocephalum L. 16 Levan 1935
triquetrum L. 18 Levan 1932
carinatum L. 24 Levan 1937 (G)
Ampeloprasum L. 32 Maude unpub.
oleraceum L. 32 Levan 1937 (G)
vineale L. 32 Levan 1931
Babingtonii Borr. 48 Maude unpub.
434. MUSCARI $x=?$
racemosum Lam. & DC. 45 Delaunay 1926
 54 Wunderlich 1937
435. SCILLA $x=6, 8, 9, 11$
autumnalis L. 24 (28) Heitz unpub.
 42 Maude unpub.
non-scripta Hoffm. & Link. 16 Darlington 1926
 [as *nutans* Sm.] Hoare 1934
verna Huds. 22 Maude unpub.
436. ORNITHOGALUM
 $x=6, 7, 8, 9$, etc.
nutans L. 16 Sprumont 1928
 30 Nakajima 1936
pyrenaicum L. 16 Greeves (T. 1931)
 32 Sprumont 1928
umbellatum L. 27 Nakajima 1936
 27, 45 Sprumont 1928
 54 Matsuura & Sutô 1935
437. LILIUM $x=12$
Martagon L. 24 Richardson 1936
pyrenaicum Gouan 24 Newton 1927
438. FRITILLARIA
 $x=9, 12, 13$
Meleagris L. 24 Darlington 1935
439. TULIPA $x=12$
sylvestris L. 48 Upcott & La Cour 1936 (S.)
440. GAGEA $x=12$
lutea Gawler 72 Matsuura & Sutô 1935
441. LLOYDIA $x=12$
serotina Reichb. 24 Newton 1927
442. COLCHICUM $x=?$
autumnale L. 42 Newton (T. 1931)
443. NARTHECIUM $x=?$
ossifragum Huds. 26 Miller 1930
 Wulff 1935
444. TOFIELDIA $x=?$
palustris Huds. 30 Miller 1930
445. PARIS $x=5$
quadrifolia L. 10 Darlington 1937
 Geitler 1936
 10, 15 Haga 1934a, b

JUNCACEAE

446. JUNCUS $x=20$
bulbosus L. 40 Timm unpub.
inflexus L. c. 40 Wulff 1937b
 [as *glaucus* Ehrh.]
maritimus Lam. c. 40
squarrosus L. c. 40 Wulff 1938
bifonius L. c. 60 Wulff 1937a
articulatus L. c. 60 Wulff 1938
 [as *lampocarpus* Ehrh.]
filiformis L. c. 80
Gerardi Lois. 80 Wulff 1937a
 and 15 other species.
447. LUZULA $x=9$
campestris DC. 18 Brenner 1922
multiflora DC. 18
arcuata Wahl.
Forsteri DC.
pallens Bess.
pilosa Willd.
spicata DC.
sylvatica Gaud.

TYPHACEAE

448. TYPHA $x=15$
latifolia L. 30 Roscoe 1927
angustifolia L. 60
 449. SPARGANIUM $x=15$
minimum Fr. 30 Wulff 1938
ramosum Curt. 30
angustifolium Michx.
neglectum Beeby
simplex Huds.

ARACEAE

450. ARUM $x=8$
maculatum L. 32 Schmucker 1925
 56, c. 84 Maude unpub.
italicum L. 64 Dangeard 1937
451. ACORUS $x=9$
Calamus L. 18 Dahl cit. Buell 1938
452. CALLA $x=9$
palustris L. 36 Dudley 1937

LEMNACEAE

453. LEMNA $x=10, 11$
minor L. 40 Blackburn 1933
polyrrhiza L. 40
triselca L. 44
gibba L. 64
 454. WOLFFIA $x=?$
arrhiza Wimm. c. 50

ALISMACEAE

455. ALISMA $x=7$
Plantago-aquatica L. 14 Heppell unpub.
lanceolatum With.
ramunculoides L.
456. ELISMA $x=?$
natans Buchenau
457. SAGITTARIA
 $x=10?, 11$
sagittifolia L. 20 Nawa 1928
 22 Lohammar 1931a
heterophylla Pursh
458. DAMASONIUM $x=?$
Alisma Mill.
459. BUTOMUS $x=?$
umbellatus L. 26 Whitaker 1934
 28, 40 Lohammar 1931b

NAIADACEAE

460. TRIGLOCHIN $x=?$
maritimum L. 48 Wulff 1937a
palustre L.
461. SCHEUCHZERIA
 $x=?$
palustris L.
462. POTAMOGETON
 $x=?$
perfoliatus L. c. 48 Wiesniewska 1931
 and 28 other species.

463. RUPPIA $x=8$		
<i>maritima</i> L.	16	Wulff 1937 a
<i>rostellata</i> Koch
464. ZANNICHELLIA $x=?$		
<i>gibberosa</i> Reichb.
<i>palustris</i> L.
<i>pedunculata</i> Reichb.
<i>polycarpa</i> Nolte
465. ZOSTERA $x=6$		
<i>marina</i> L.	12	Wulff 1937 a
<i>nana</i> Roth	12	"
466. NAIAS $x=6$		
<i>marina</i> L.	12	Lewitzky 1931 a
		Wulff 1937 a
<i>flexilis</i> Rostk. & Schmidt
<i>graminea</i> Delile

ERIOCAULACEAE

467. ERIOCAULON $x=?$		
<i>septangulare</i> With.

CYPERACEAE

468. CYPERUS $x=?$		
<i>fuscus</i> L.
<i>longus</i> L.
469. ELEOCHARIS		
<i>palustris</i> R. & S. $x=5, 8$, etc.		
[as <i>Scirpus palustris</i> L.]	16, 38	Håkansson 1929 b
	10	Avdulov 1931
	10, 16	Lewitzky (l. 1936)
<i>multicaulis</i> Sm.	20	Håkansson 1928
<i>uniglumis</i> Schultes	32	Piech 1928 a, b
[as <i>Heleocharis uniglumis</i>]	46	Håkansson 1928
<i>acicularis</i> R. & S.	56	Hicks 1929
470. SCIRPUS $x=5, 13$, etc.		
<i>parvulus</i> Roem. & Schult.	10	Wulff 1937 a
<i>setaceus</i> L.	26	Håkansson 1928
<i>rufus</i> Schrad.	40	Wulff 1937 a
<i>lacustris</i> L.	42	Kostrionkoff 1930
	38, 40, 42	Tanaka 1938
<i>Tabernaemontani</i> Gmel.	42	Håkansson 1928
	c. 44	Wulff 1938
<i>compressus</i> Pers.	44	Håkansson 1928
<i>syloaticus</i> L.	62	"
		Avdulov 1931
<i>americanus</i> Pers.	76, 100-128	Hicks 1928
	c. 80	Wulff 1937 a
<i>maritimus</i> L.	104	Håkansson 1928
	86	Blackburn 1933
<i>caespitosus</i> L.
<i>filiformis</i> Savi
<i>fluitans</i> L.
<i>Holoschoenus</i> L.
<i>pauciflorus</i> Lightf.
<i>triqueter</i> L.
471. ERIOPHORUM $x=29$		
<i>vaginatum</i> L.	58	Håkansson 1928
<i>alpinum</i> L.
<i>angustifolium</i> Roth
<i>gracile</i> Koch & Roth
<i>latifolium</i> Hoppe
472. RHYNCHOSPORA $x=?$		
<i>alba</i> Vahl
<i>fusca</i> Ait.
473. SCHOENUS $x=?$		
<i>ferrugineus</i> L.
<i>nigricans</i> L.
474. CLADIUM $x=?$		
<i>Mariscus</i> R. Br.
475. KOBRESIA $x=?$		
<i>bipartita</i> Dal. Tor.
476. CAREX $x=8, 9$, etc.		
<i>pilulifera</i> L.	18	Heilborn 1924
<i>ericetorum</i> Poll.	30	"
<i>panicea</i> L.	32	"
<i>vaginata</i> Tausch	32	"
(<i>sparsiflora</i> Steud.)		
<i>atrofusca</i> Schkuhr	36	Heilborn 1928
<i>montana</i> L.	38	Heilborn 1924
<i>Davalliana</i> Sm.	46	Heilborn 1937

<i>ornithopoda</i> Willd.	c. 46	Heilborn 1924
<i>tomentosa</i> L.	48	"
<i>rupestris</i> Bell.	50	"
<i>digitata</i> Bell.	52	"
<i>dioica</i> L.	52	"
<i>atrata</i> L.	54	"
<i>capillaris</i> L.	54	"
<i>canescens</i> L.	56	"
<i>elongata</i> L.	c. 56	"
<i>fulva</i> Host.	56	Heilborn 1928
(<i>Hornschuchiana</i> Hoppe)		
<i>Halleri</i> Gunn.	56	Heilborn 1924
(<i>alpina</i> Sw.)		
<i>contigua</i> Hoppe	c. 58	"
<i>divulsa</i> Stokes	58	"
<i>extensa</i> Good.	c. 60	Wulff 1937 a
<i>caryophyllea</i> Latour.	62	Heilborn 1924
<i>disticha</i> Huds.	c. 62	"
<i>remota</i> L.	62	"
<i>arenaria</i> L.	c. 64	Wulff 1937 b
<i>pallesens</i> L.	64	Heilborn 1924
<i>paradoxa</i> Willd.	64	"
<i>Pseudo-Cyperus</i> L.	66	"
<i>lepidocarpa</i> Tausch.	68	"
<i>punctata</i> Gaud.	68	"
<i>vilpina</i> L.	68	"
<i>Oederi</i> Retz.	70	Heilborn 1928
<i>rigida</i> Good.	70	"
<i>riparia</i> Curt.	72	Heilborn 1924
<i>aquatilis</i> Wahl.	c. 74	Stout 1913
<i>distans</i> L.	74	Wulff 1937 a
<i>fusca</i> All.	c. 74	Heilborn 1924
[as <i>Buxbaumii</i> Wahl.]		
<i>diversicolor</i> Crantz	76	"
[as <i>glauca</i> Scop.]		
<i>inflata</i> Huds.	76	"
[as <i>rostrata</i> Stokes]		
<i>Hudsonii</i> A. Benn	80	"
<i>saxatilis</i> L.	80 (82)	Heilborn 1928
<i>vesicaria</i> L.	82	Heilborn 1924
<i>Goodenowii</i> Gay	84	"
<i>gracilis</i> Curt.	84	"
<i>hirta</i> L.	112	"
and 34 other species.		

GRAMINEAE

477A. DIGITARIA $x=9$		
<i>sanguinalis</i> Scop.	36	Avdulov 1928
[as <i>Panicum sanguinale</i>]		
<i>Ischnaeum</i> Muhl.
(<i>Panicum lineare</i> Auct. non L.)		
477B. ECHINOCHLOA $x=?$		
<i>crus-galli</i> Beauv.	42, 56	Church 1929 b
	54	Avdulov 1928
	48?	Rau 1929
478. SETARIA $x=9$		
<i>viridis</i> Beauv.	18	Avdulov 1928
		Kishimoto 1938
<i>glauca</i> Beauv.	36	Avdulov 1928
		Kishimoto 1938
<i>verticillata</i> Beauv.	36	Avdulov 1928
479. SPARTINA $x=7$		
<i>stricta</i> Roth	56	Huskings 1931
<i>alterniflora</i> Loisel.	70	"
<i>Townsendii</i> H. & J. Groves	126	"
(<i>S. alterniflora</i> \times <i>S. stricta</i>)		
480. LEERSIA $x=?$		
<i>oryzoides</i> Sw.	48	Ramanujam 1938
481. PHALARIS $x=6, 7$		
<i>canariensis</i> L.	12	Parthasarathy 1938
	28	Nakajima 1933
<i>paradoxa</i> L.	14	Parthasarathy 1938
<i>arundinacea</i> L.	28	Nielsen & Humphrey 1937
		Hunter 1934
<i>minor</i> Retz.	28	Parthasarathy 1938
482. ANTHOXANTHUM $x=5$		
<i>aristatum</i> Boiss.*	10	Avdulov 1928
<i>odoratum</i> L.	20+6ff	Hunter 1934
		Parthasarathy 1938
483. HIEROCHLOE $x=7$		
<i>odorata</i> Beauv.	28	Kattermann 1932

* The British plants so named are distinct from true *A. aristatum* Boiss. and are now known as *A. Puellii* Lec. & Lam.

484. ALOPECURUS $x=7$			
<i>bulbosus</i> Gouan.	14	Maude unpub.	
<i>aequalis</i> Sobol.	14	Kattermann 1930	
[<i>as fulvus</i> Sm.]			
<i>myosuroides</i> Huds.	14	"	
<i>geniculatus</i> L.	28	"	
<i>pratensis</i> L.	28	Rancken 1934	
<i>alpinus</i> Sm.*	
485. MILIUM $x=7$			
<i>effusum</i> L.	28	Avdulov 1928	
<i>scabrum</i> Rich.	
486. PHLEUM $x=7$			
<i>arenarium</i> L.	14	Wulff 1937a	
<i>phleoides</i> (L.) Karst.	14	Avdulov 1931	
[<i>as Boehmeri</i> Wibel]		Hunter 1934	
<i>nodosum</i> L.	14	Nordenskiöld 1937	
(<i>pratense</i> var. <i>nodosum</i> Huds.)		(G)	
<i>alpinum</i> L.	28	Gregor & Sansome	
		1930 (G, S)	
		Nordenskiöld 1937	
		(G)	
<i>pratense</i> L.	42	Gregor & Sansome	
		1930 (G, S)	
487. MIBORA $x=7$			
<i>minima</i> Desv.	14	Avdulov 1928	
[<i>as verna</i> Beauv.]			
488. AGROSTIS $x=7$			
<i>setacea</i> Curt.	14	Maude unpub.	
<i>conina</i> L.	14	Sokolovskaja 1938	
	28	Wulff 1937b	
<i>tenuis</i> Sibth.	28	Avdulov 1928	
(<i>vulgaris</i> With.)			
<i>semitruncata</i> C. Christ	28	Avdulov 1931	
[<i>as verticillata</i> Vill.]			
<i>alba</i> Auct.	28	Sokolovskaja 1938	
(<i>stolonifera</i> L.)	42	Avdulov 1928	
	28, 56	Church 1936	
<i>nigra</i> With.	42	Maude unpub.	
489. POLYPOGON $x=7$			
<i>montepeliensis</i> Desf.	28	Avdulov 1928	
490. CALAMAGROSTIS			
<i>epigejos</i> Roth.	$x=7$	Westergård unpub.	
	28, 56	Avdulov 1931	
<i>neglecta</i> Gaertn.	c. 70	"	
<i>lanceolata</i> Roth.	c. 70	"	
	
491. GASTRIDIMUM $x=?$			
<i>ventricosum</i> Schinz & Thell.	
(<i>lindigerum</i> Gaud.)			
492. APERA $x=7$			
<i>Spica-Venti</i> Beauv.	14	Avdulov 1931	
<i>interrupta</i> Beauv.	14	Maude unpub.	
493. AMMOPHILA $x=7$			
<i>arenaria</i> Link	28	Wulff 1937b	
<i>baltica</i> Link	42	Westergård unpub.	
494. LAGURUS $x=7$			
<i>ovatus</i> L.	14	Avdulov 1931	
495. AIRA $x=7$			
<i>caryophyllea</i> L.	14	Wulff 1937b	
<i>praecox</i> L.	14	Maude unpub.	
		Hagerup 1939	
496. CORYNEPHORUS $x=?$			
<i>canescens</i> Beauv.	$x=?$		
[<i>as Aira canescens</i>]	14	Avdulov 1931	
497. DESCHAMPSIA $x=7$			
<i>setacea</i> Hack.	14	Maude unpub.	
<i>caespitosa</i> Beauv.	28	Hagerup 1939	
		Avdulov 1928	
		Nielsen & Hum-	
		phrey 1937	
<i>flexuosa</i> Trin.	28	Hagerup 1939	
<i>alpina</i> R. & S.	39, 41, 49	Stählin 1929	
	56	Flovik 1938	
	49	Hagerup 1939	
		Maude unpub.	
498. HOLCUS $x=7$			
<i>lanatus</i> L.	14	Avdulov 1928	
<i>mollis</i> L.	14	Stählin 1929	
		"	
499. TRISETUM $x=6$			
<i>flavescens</i> Beauv.	24	Avdulov 1931	
		Kattermann (T. 1936)	
500. AVENA $x=7, 8$			
<i>strigosa</i> Schreb.	14	Emme 1932	
<i>pubescens</i> Huds.	16	Kattermann (T. 1936)	
<i>fatua</i> L.	42	Emme 1932	
<i>pratensis</i> L.	42	Huskins 1927 (G)	
501. ARRHENATHERUM			
<i>elatius</i> J. & C. Presl	$x=7$	Maude unpub.	
(<i>A. avenaceum</i> Beauv.)	28	Jenkin 1931 (G)	
<i>tuberosum</i> Schultz	..		
(<i>A. avenaceum</i> var. <i>bulbosum</i>)			
502. CYNODON $x=7, 9, 15$			
<i>Dactylon</i> (L.) Pers.	36	Kattermann 1931	
	30	Avdulov 1931	
503. SIEGLINGIA (apo?)			
<i>decumbens</i> Bernh.	$x=?$		
504. PHRAGMITES			
<i>communis</i> Trin.	$x=7$	Maude unpub.	
	48		
505. SESLERIA			
<i>caerulea</i> Ard.	$x=7$	Avdulov 1934	
	28		
506. CYNOSURUS $x=7$			
<i>cristatus</i> L.	14	Kattermann 1930	
<i>echinatus</i> L.	14		
		Avdulov 1928	
		Stählin 1929	
507. KOELERIA $x=7$			
<i>glauca</i> DC.	14	Avdulov 1928	
<i>gracilis</i> Pers.	28, 30	Maude unpub.	
<i>vallesiana</i> Bertol.	42	"	
508. MOLINIA $x=9$			
<i>caerulea</i> Moench.	36	Jefferies (T. 1936)	
509. CATABROSA $x=10$			
<i>aquatica</i> Beauv.	20	Avdulov 1931	
510. MELICA $x=9$			
<i>nutans</i> L.	18	Kattermann 1930	
		Avdulov 1931	
<i>uniflora</i> Retz.	
511. DACTYLIS $x=7$			
<i>glomerata</i> L.	28	Müntzing 1937	
512. BRIZA $x=5, 7$			
<i>minor</i> L.	10	Avdulov 1931	
		Kattermann 1933	
<i>maxima</i> L.	14	Avdulov 1931	
		Kattermann 1933	
<i>media</i> L.	14	Avdulov 1931	
		Kattermann 1933	
513. POA (apo) $x=7$			
<i>Chaixii</i> Vill.	14	Åkerberg 1936	
<i>trivialis</i> L.	14	Avdulov 1931	
<i>annua</i> L.	28	Müntzing 1932	
		Avdulov 1931	
		Müntzing 1932	
		Kattermann 1930	
		Avdulov 1931	
<i>bulbosa</i> L.	28	Armstrong 1937	
<i>nemoralis</i> L.	28	Nannfeldt 1937 (S)	
	42	Armstrong 1937	
	56	Avdulov 1931	
<i>alpina</i> L.	42	Müntzing 1932	
		Stählin 1929	
<i>Balfouri</i> Parn.	32-34	Armstrong 1937	
<i>compressa</i> L.	42	Maude unpub.	
	42	Avdulov 1931	
	42, 56	Müntzing 1932	
<i>glauca</i> Vahl.	70	Armstrong 1937	
<i>irrigata</i> Lindm.	90	Avdulov 1928	
<i>pratensis</i> L.	28, 56, 70	Åkerberg unpub.	
	49, 64, 85	Avdulov 1931	
	70	Müntzing 1932	
	66, 67,	Nakajima 1933	
	67 + ff.		
<i>flexuosa</i> Sm.	50-120	Rancken 1934	
<i>remotiflora</i> Murb.	..	Åkerberg unpub.	
	

* *A. alpinus* var. *elatius* Korn., a non-British variety, has $2n=70$ (Avdulov, 1931).

514. GLYCERIA $\alpha=7$					
<i>declinata</i> Bréb.	20	Maude unpub.	<i>erectus</i> Huds.	56	Kattermann 1931
<i>maxima</i> Holmb.	28	Stählin 1929	<i>rigidus</i> Roth.	56	Avdulov 1931
[as <i>aquatica</i> Wahl.]			[as <i>maximus</i> Desf.]		Stählin 1929
<i>fluitans</i> R.Br.	28		<i>commutatus</i> Schrad.
<i>plicata</i> Fries	40	Maude unpub.	<i>molliformis</i> Lloyd
	28	Stählin 1929			
	40	Maude unpub.	517. BRACHYPODIUM		
514A. PUCCINELLIA (apo?)			<i>sylvaticum</i> Beauv.	$\alpha=7, 9$	Avdulov 1928
$\alpha=7$			<i>pinnatum</i> Beauv.	18	Kattermann 1930
<i>distans</i> Parl.	28	Stählin 1929	518. LOLIUM $\alpha=7$		Jenkin 1933 (G, S)
[as <i>Atropis distans</i>]					Jenkin & Thomas
[<i>Glyceria distans</i> Wahl.]			<i>multiflorum</i> Lam.	14	1938 (G)
<i>maritima</i> Parl.	63	Maude unpub.	<i>perenne</i> L.	14	Peto 1933
[<i>G. maritima</i> Mert. & Koch]					Kattermann 1930
<i>fasciculata</i> Bickn.	<i>renotum</i> Schrank	14	Peto 1933
[<i>G. Borreri</i> Bab.]			[as <i>linicola</i> Sonder]	14	Thomas 1936 (G)
<i>rupestris</i> Fern. & Weath.	<i>temulentum</i> L.	14	Thomas unpub.
[<i>G. rupestris</i> E. S. Marshall]					Faworski 1927
515. FESTUCA* $\alpha=7$		Jenkin 1933 (G, S)			Faworski 1927
<i>bromoides</i> L.	14	Stählin 1929	519. AGROPYRON $\alpha=7$		Avdulov 1931
<i>capitata</i> Lam.	14	Thomas unpub.	<i>caninum</i> Beauv.	28	Simonet 1935
<i>pratensis</i> Huds.	14	Jenkin 1933 (G, S)	<i>juncens</i> Beauv.	28	Simonet 1934b
		Rancken 1934	<i>pungens</i> R. & S.	42	
<i>rigida</i> Knuth	14	Maude unpub.	<i>repens</i> Beauv.	42	Avdulov
[as <i>Scleropoa rigida</i>]		Avdulov 1928		42, 28	Avdulov 1931
<i>ciliata</i> Danth.	28	Avdulov 1931	<i>Donianum</i> F. B. White	34, 35, 42	Peto 1930
[<i>F. Danthonii</i> Asch. & Gr.]			
<i>glauca</i> Lam.	28	Stählin 1929	520A. PHOLIURUS $\alpha=7, 9$		
[<i>F. ovina</i> var. <i>glauca</i>]			<i>filiformis</i> Schinz & Thell.	14	Avdulov 1928
<i>ovina</i> L. (viviparous)	14	Turesson 1938	[as <i>Lepturus filiformis</i> Trin.]	36	Wulff 1937a
	28, 42	Jenkin 1933 (G, S)	<i>incurvus</i> Schinz & Thell.	36	Avdulov 1928
	28	Church 1929a	[as <i>L. incurvatus</i> Trin.]		
<i>arundinacea</i> Schreb.	42	Jenkin 1933 (G, S)	521. NARDUS (apo)		Coulon, cit. Schnarf
<i>heterophylla</i> Lam.	42	Lewitzky & Kuzmina 1927	<i>stricta</i> L.	26	1929
[as <i>F. rubra</i> subsp. <i>heterophylla</i>]					Avdulov 1928
<i>fallax</i> Thuill.	42	Maude unpub.	522. HORDEUM $\alpha=7$		
<i>longifolia</i> Thuill.	42		<i>maritimum</i> Huds.	14	Ghimpu 1930
<i>sylvatica</i> Vill.	42	Stählin 1929	[as <i>maritimum</i> With.]	14	Wulff 1937a
<i>gigantea</i> Vill.	42		<i>murinum</i> L.	14	Ghimpu 1930
[as <i>Eriomys giganteus</i> L.]			<i>europaeum</i> All.	28	Stählin 1929
<i>rubra</i> L. (viviparous)	42	Church 1929a	[as <i>sylvaticum</i> Huds.]		
	28, 42	Jenkin 1933 (G, S)	<i>nodosum</i> L.	42	Griffie 1927
	56	Thomas unpub.	523. ELYMUS $\alpha=?$		
<i>uniglumis</i> Sol.	42	Maude unpub.	<i>arenarius</i> L.	56	Stählin 1929
<i>juncifolia</i> St Am.			
<i>rotboelliioides</i> Kunth.			
<i>supina</i> Schur.			
516. BROMUS $\alpha=7$					
<i>arvensis</i> L.	14	Avdulov 1928			
		Stählin 1929			
<i>ramosus</i> Huds.	14	"			
<i>sterilis</i> L.	14	"			
<i>tectorum</i> L.	14	Avdulov 1928			
		Stählin 1929			
<i>hordeaceus</i> L.	28	Avdulov 1928			
[<i>B. mollis</i>]		Stählin 1929			
<i>interruptus</i> Druce	28	Maude unpub.			
<i>lepidus</i> Holmb.	28	"			
[<i>britannicus</i> F. A. Williams]					
<i>racemosus</i> L.	28	Avdulov 1931			
<i>secalinus</i> L.	28	Nakajima 1931			
		Stählin 1929			
<i>madritensis</i> L.	42	Avdulov 1931			
	28				

* The following species are now placed in the genus *Vulpia*: *ambigua*, *bromoides*, *ciliata*, *myuros* and *uniglumis*.

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EXPERIMENTAL TAXONOMY

III. CORRELATION OF CHARACTERS WITHIN A POPULATION

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INTRODUCTION

WHEN populations of plants are grown under the considerable evenness of environment of an experimental garden, comparable measurements of various characters can be treated statistically for the determination of mean difference and variance. Common observation then suggests that some of the measurements are apparently correlated, and in particular that they tend to vary in accordance with the size of the plant. It is of interest to ascertain to what extent these correlations actually exist, how far they may be due to growth, and how far to other causes. From this viewpoint it is then possible to assess the suitability of characters for taxonomic purposes, and to examine the meaning of the term "correlation" when used to describe associated variations in the forms of characters as displayed by taxonomic units.

Dr Gregor's plantain material provides a superabundance of data concerning some thirty-eight characters recorded more or less throughout fifty populations. Several lines of investigation are therefore possible: (1) the examination of correlations of characters on organs of the same plant, e.g. measurements of flower parts on a series of scapes; (2) the examination of all the interrelationships of a number of characters in a few populations; (3) the tracing of the behaviour of a few characters throughout a series of populations; and (4) the correlation of mean values of the

various populations. The second course, a study of correlation *within the population*, was chosen because not only would it present a picture of the complex conditions governing the appearance of characters, but it might also raise questions worthy of further investigation. Twenty characters were therefore examined in three populations.

DESCRIPTION OF CHARACTERS

With one exception the characters were defined in the first paper of this series (Gregor *et al.* 1936). They may be arranged under five headings and summarized as follows:

I. Time of flowering is denoted by (1) *Flowering grade*, an arbitrary series of eleven classes, indicating progress in flowering from grade 1, where it has not commenced, to grade 11, where it has ceased. As all the plants are observed on the same date, the higher the grade reached the earlier the plant.

II. The arrangement of flowers is noted by (2) *Spike density*, which is a count of the number of floral attachments on the middle centimetre of the spike, and hence the greater the number the more compact is the spike.

III. Habit of growth is represented by a grade and two ratios of measurements. (3) *Habit grade* is an arbitrary division into five classes of scape position, of which grade 1 is decumbent with spikes restricted to the periphery, while the others have spikes evenly distributed, and rise from grade 2 decumbent to grade 5 erect. The ratios (4) *Scape spread: height* and (5) *Leaf spread: height* also denote habit of growth, and are compiled from measurements of spread across the greatest diameter, and of height from ground level to the highest point.

IV. The size of the plant is pictured as an imaginary erect cylinder, (6) *Scape volume*, occupying the height and the circle of spread of the scape system (Gregor, 1938). This is merely a rough indication of the space occupied, for the forms vary indefinitely.

V. For the size and shape of organs, twelve measurements and two derived ratios, called indices, are adopted. The anther is measured for total length and for the length of its subulate tip, the indices of bract and sepal are the ratios of length: breadth in these organs; and scape length includes spike and rachis, otherwise the titles of the characters are self-explanatory (nos. 7-20).

The methods of selective sampling were discussed in the first paper (Gregor *et al.* 1936), and intraclass correlations were employed to test their validity (*ibid.* Table III). In Table IV of that paper comparisons were made of *organic* and *cross-homotypic* correlations after the manner of Pearson (1901). If, in each plant, a pair of measurements such as length and breadth be made on one leaf, there will be usually a slightly higher correlation throughout the population than if length be taken on one leaf and breadth on another very similar (homotypic) leaf of the same plant. The discrepancy is generally negligible, but since it might occasionally affect a result, organic correlations are preferable whenever it is possible to measure both characters on a selected organ. This accorded with the sampling technique already employed.

MATERIAL

The samples of populations of *Plantago* spp. were grown in an experimental garden near Edinburgh. Each consisted of 102 plants, upon which twenty characters were recorded. Thus there should be 102 pairs of characters available for each correlation, which allows levels of significance at 0.1946 when $P=0.05$, and at 0.2540 when $P=0.01$ (Fisher, 1932, Table V(A)). Roughly speaking, coefficients of over ± 0.2 are worthy of consideration, though some of them will be accidental.

These conditions obtained in samples of two populations named PMN 63 and PMN 44, which were grown in 1932-3. The records of a third, PMN 45, were less complete, partly because several of the plants died during the summer of observation in 1932; but mainly because it was then the practice to measure anthers, bracts and sepals on fifty or sixty-five plants only, while the breadth of the sepal and consequently the index were not then ascertained. The number of pairs available for each correlation in PMN 45 can be obtained from Table XXII, but, to give a general indication of significance, all coefficients having a probability of 0.05 or less have been printed in bolder type in the tables.

PMN 63 was a sample of *Plantago serpentina* All. collected by Dr Gregor at an altitude of 4050 ft. at Seefeld in Austria. This population has been found to be tetraploid (Bennett, unpublished), whereas *P. maritima* L. is diploid ($n=6$). PMN 44 was a sample of *P. maritima* L. from a river bank habitat at Struan, in Perthshire, altitude 450 ft.; while PMN 45 was also *P. maritima* from a coastal habitat at Brancaster, Norfolk. These populations were chosen for study as being representative of the collection. The cytological distinctions were not then known, but some of the characters of *P. serpentina* seemed to offer small contrasts to those of the other populations, though all the characters showed continuous variation and the ranges overlapped.

TREATMENT OF DATA

The direct correlations for all possible pairs of characters were calculated and are shown in the tables at least once and in most cases twice under the headings of the various characters. Positive correlations among the various size characters were apparent, especially in PMN 45 where they were of considerable magnitude. Three characters were therefore chosen as representing general trends of growth in the plant, and were used in partial correlations to eliminate growth effects from the other correlations. Of these, the character scape length is measured when growth of all parts except the seed has practically ceased, and it is representative, therefore, of the mature state of the plant as well as of the scape region. But since the length alone might have some genetical significance not altogether identical with general growth, scape thickness was employed as a check. Generally the effect of this character was similar to, or not so marked as, that of scape length in the partials, but in a few instances it showed some particular bearing on other correlations. The third character used for elimination in partials was an early measurement of leaf length. Taken before flowering commenced, this might be expected to represent

the peak of vegetative activity for the second season of growth. In practice, however, the conditions obtaining in each population on 15 May, when the measurement was made, were not necessarily comparable, and the effects of eliminating leaf length differed greatly in the three populations. Other characters were eliminated in special circumstances (Table II).

Limitations of space must be made the excuse for some treatments which may be troublesome to the reader. First, the names of the characters are contracted to symbols in the tables, and secondly, correlations that have been discussed under the heading of one character may be omitted from the account of the other. Thirdly, it is not possible to print all the scape thickness partial correlations. The following contractions have been adopted:

A , anther	G , grade	S , spread
AT , anther tip	H , height	Sc , scape
B , breadth	Hb , habit	Sep , sepal
Br , bract	Ix , index	Sp , spike
D , density	L , length	Th , thickness
Fl , flowering	Lf , leaf	V , volume

CORRELATION BETWEEN CHARACTERS

I. *Time of flowering*

(1) *Flowering grade, FIG* (Table I). The complete figures for correlations with flowering grade are given in Table I. For each population there is a column showing direct coefficients of correlation and three columns giving modified coefficients after scape length, scape thickness and leaf length have been respectively eliminated. The relationships between time of flowering and the other characters are somewhat vague and when significant correlations occur in one population they may be absent from another. Examining the results, it may be seen that:

(a) There is no relationship with spike density (**SpD**).

(b) There is some connexion between time of flowering and habit of growth in PMN 63 and PMN 44, but not in PMN 45. The strongest correlation is with the ratio scape spread : height (**ScS : H**), where the more spreading types seem to be earlier, while the same effect is seen less distinctly with habit grade (**HbG**), wherein the method of grading gives a negative sign. The ratio leaf spread : height shows a similar tendency for procumbency to be correlated with earliness, but in PMN 63 the partial correlation shows this to be due to common relationships with length of leaf (**LfL**).

(c) There is no relationship between time of flowering and size of floral parts in PMN 44, but in PMN 63 there are inverse tendencies, especially when leaf length has been eliminated. A hypothetical explanation might involve the condition of the plants on the dates when the anthers, bracts and sepals are measured. For example the anthers which are exerted on a certain date may occur on late spikes of the earlier flowering plants and on the first spikes of plants which are only then commencing to flower. Some small experiments have suggested that the anthers exerted on the first day or two may be distinctly larger than the fairly

constant size maintained during the following fortnight. In PMN 45 the anther lengths show the negative correlation, but the bracts and sepals are positively correlated with time of flowering. Here time of flowering seems to be strongly connected with general growth, which, as will be seen later, controls the size of bract and sepal to a remarkable extent in this population.

(d) If a measurement be made on an organ which is actively growing, it is likely that the plants with largest variates may be precocious and flower relatively early. The early observations on the leaf are most likely to show this effect, and hence it is not surprising to find positive correlations with them. In PMN 63 and PMN 45, leaf length is the most important of these, since its elimination reduces the others to insignificance in the partials; indeed the leaf length of PMN 45 also effectively disposes of the whole series of positive correlations. In PMN 44, however, leaf length has no connexion with time of flowering, possibly because growth of the leaf had reached a stage where it no longer gauged the state of development. It is convenient to adopt Lysenko's distinction that *growth* implies increase in weight and volume of a plant at any particular stage, whereas *development* is concerned with passage through stages in the life history (Whyte & Hudson, 1933). The negative correlation between flowering grade and scape length in PMN 44 is peculiar; it may be a reflexion of habit, since there is a negative correlation between scape length and prostrateness (see § 3), but if so the corresponding effect is not apparent in PMN 63.

II. Arrangement of flowers

(2) *Spike density, SpD* (Table II). The density of the arrangement of flowers on the spike is independent of flowering time, growth habit, leaf size and scape dimensions. There is, however, an inverse correlation with spike length, indicating that short spikes tend to be more compact; but in no population did any segregation into long-lax and short-compact types occur, such as has been obtained by hybridizing *P. juncooides* Lam. \times *P. oliganthos* R. & S. (Gregor, unpublished).

Correlations also occur between spike density and various dimensions of the sepal, the bract and, in PMN 44, the anther. These are also negative, suggesting that the more crowded flowers have smaller parts, but they are not strong and occur irregularly. When it was found that the bract breadth of PMN 45 was correlated while the length was not, some special relationship was suspected, e.g. broader flowers on lax spikes, so several other populations were examined with the following results:

Correlation	PMN 67	PMN 62	PMN 39	PMN 38	PMN 60	PMN 66
SpD v. BrL	-0.21	-0.16	-0.09	-0.08	-0.16	+0.11
SpD v. BrB	-0.26	-0.25	-0.21	-0.11	-0.03	-0.02

It will be seen that the correlations are at best barely significant, and that they occur as frequently with bract length as with the breadth. When the index of length : breadth shows a correlation it is a reflexion of one or both measurements. If the length correlation is the greater the sign is unchanged, but if the breadth is more important the sign of the index correlation is reversed (see Discussion).

Table II. *Correlations with spike density*

Character	PMN 63				PMN 44				PMN 45			
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	-ScL	Eliminating		Direct coeff.	-ScL
		-ScL	-SpL		-ScL	-SpL			-ScL	-SpL		
FIG	+0.110	+0.111	+0.134	+0.053	-0.123	-0.102	-0.129	+0.014	+0.028	+0.026	-0.003	+0.026
HbG	+0.0	+0.024	+0.037	+0.006	-0.011	+0.035	+0.026	+0.185	+0.179	+0.180	+0.169	+0.180
ScS : H	+0.017	-0.001	-0.037	-0.045	+0.038	-0.016	-0.037	-0.123	-0.124	-0.120	-0.121	-0.120
LFS : H	+0.037	+0.046	+0.053	+0.053	-0.026	-0.039	-0.028	-0.089	-0.080	-0.090	-0.092	-0.090
ScV	-0.187	-0.194	-0.107	-0.144	-0.044	+0.013	-0.045	+0.058	+0.071	+0.041	-0.009	+0.041
AL	-0.143	-0.132	-0.096	-0.092	-0.266	-0.248	-0.237	+0.101	+0.159	+0.168	+0.167	+0.168
ATL	+0.091	+0.090	+0.074	+0.080	-0.236	-0.253	-0.199	+0.086	+0.073	+0.093	+0.086	+0.093
SepL	-0.302	-0.206	-0.282	-0.209	-0.276	-0.234	-0.142	-0.051	-0.003	-0.037	-0.067	-0.037
SepB	-0.182	-0.166	-0.134	-0.151	-0.110	-0.005	-0.047	—	—	—	—	—
SepLx	-0.035	-0.062	-0.071	+0.023	-0.102	-0.106	-0.027	—	—	—	—	—
BrL	-0.250	-0.239	-0.168	—	-0.253	-0.213	—	-0.041	+0.017	—	-0.060	-0.041
BrB	-0.218	-0.206	-0.185	-0.104	-0.131	-0.068	-0.007	-0.305	-0.284	-0.337	-0.282	-0.337
Brix	-0.056	-0.046	-0.025	+0.087	-0.198	-0.200	-0.063	+0.313	+0.320	+0.389	+0.208	+0.389
ScL	-0.076	—	+0.088	+0.018	-0.209	—	-0.156	—	+0.043	-0.001	-0.044	-0.001
SpL	-0.202	-0.206	—	-0.131	-0.383	-0.329	-0.334	-0.103	—	-0.086	-0.104	-0.103
ScTh	-0.125	-0.100	+0.035	-0.056	-0.134	-0.059	-0.070	+0.073	+0.148	+0.077	+0.014	+0.073
LfL	-0.050	-0.012	+0.042	-0.004	-0.026	+0.073	+0.054	+0.001	+0.046	+0.025	-0.026	+0.001
LfB	-0.117	-0.095	-0.024	-0.093	-0.034	+0.003	-0.014	+0.066	+0.089	+0.060	+0.027	+0.066
LfTh	-0.081	-0.067	-0.029	-0.057	-0.065	-0.078	-0.016	-0.009	+0.018	+0.003	-0.029	+0.003

None of the normal partial eliminations had any effect on the correlations with spike density, so that others eliminating bract length and spike length have been inserted. The most noticeable result of this is the reduction to insignificance of a correlation with scape length in PMN 44.

III. *Habit of growth*

(3) *Habit grade, HbG* (Table III). The correlation between grades of scape habit and the ratio scape spread : height is as strong as might be expected, the negative sign being due to the method of recording. Another definite correlation appears between habit and length of scape. Erectness is associated with greater length, and although the coefficients are small this is a correlation of some interest, for it is distinct from general growth. Erect types of plantain are adapted to compete with strongly growing vegetation, and hence length is a character of value; prostrate types on the other hand may have to withstand wind or grazing, for which length might be disadvantageous.

Table III. *Correlations with habit grade*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		- ScL	- LfL		- ScL	- LfL		- ScL	- LfL
ScS : H	-0.400	-0.353	-0.418	-0.746	-0.726	-0.743	-0.592	-0.597	-0.608
LfS : H	-0.106	-0.148	-0.201	-0.456	-0.459	-0.444	+0.122	+0.106	+0.135
ScV	+0.008	-0.333	-0.119	-0.176	-0.526	-0.128	+0.225	+0.040	+0.483
AL	+0.012	-0.046	±0.0	+0.007	-0.031	-0.004	-0.075	-0.031	-0.091
ATL	-0.035	-0.030	-0.031	+0.020	+0.037	+0.007	+0.062	+0.064	+0.068
SepL	+0.051	+0.015	+0.036	+0.110	+0.035	+0.172	-0.181	-0.385	-0.167
SepB	-0.012	-0.155	-0.029	+0.236	+0.180	+0.254	—	—	—
SepIx	+0.022	+0.127	+0.030	-0.126	-0.130	-0.102	—	—	—
BrL	+0.026	-0.098	-0.009	+0.142	+0.075	+0.191	+0.139	-0.048	+0.275
BrB	-0.052	-0.140	-0.066	+0.094	-0.003	+0.140	-0.045	-0.207	-0.009
BrIx	+0.022	-0.026	-0.005	+0.096	+0.096	+0.106	+0.025	-0.176	+0.092
ScL	+0.303	—	+0.249	+0.296	—	+0.392	+0.237	—	+0.469
SpL	+0.180	-0.041	+0.115	+0.032	-0.151	+0.069	+0.073	-0.141	+0.146
ScTh	+0.024	-0.163	-0.062	+0.265	+0.171	+0.307	+0.121	-0.092	+0.219
LfL	+0.180	+0.025	—	-0.126	-0.295	—	-0.072	-0.422	—
LfB	-0.081	-0.228	-0.194	+0.053	+0.002	+0.129	+0.217	+0.097	+0.433
LfTh	-0.024	-0.090	-0.095	-0.094	-0.082	-0.051	+0.144	+0.041	+0.221

The other correlations are irregular in occurrence. In PMN 44, the habits of scape and leaf (LfS : H) run parallel (see § 5) and scape thickness shows a similar correlation to that of scape length. The positive correlation with sepal breadth, however, appears to be accidental, as may also be the correlation with leaf breadth in PMN 45. The positive correlation with scape length affects the partials from which that character has been eliminated, and there are swings in a negative direction. On the other hand, in PMN 44 and PMN 45 the inverse correlations with leaf length, insignificant though they are, tend to enhance the leaf-length partials, for this character is positively correlated with most of the others.

(4) *Scape spread : height, ScS : H* (Table IV). The correlations of this ratio are somewhat confused, but PMN 45 may be distinguished from the other two populations because it shows no correlations except with habit grade. Four sorts of correlation are found in PMN 63 and PMN 44.

Table IV. *Correlations with the ratio scape spread : height*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		- ScL	- LfL		- ScL	- LfL		- ScL	- LfL
HbG	-0.400	-0.353	-0.418	-0.746	-0.726	-0.743	-0.592	-0.597	-0.608
LfS : H	+0.213	+0.249	+0.208	+0.439	+0.439	+0.431	+0.048	+0.053	+0.069
ScV	+0.306	+0.736	+0.334	+0.304	+0.658	+0.303	-0.006	+0.083	+0.164
AL	-0.182	-0.145	-0.187	+0.017	+0.050	+0.025	-0.071	-0.083	-0.098
ATL	-0.026	-0.032	-0.025	-0.016	-0.031	-0.007	-0.117	+0.117	-0.109
SepL	-0.216	-0.194	-0.222	-0.194	-0.136	-0.251	+0.108	+0.167	+0.193
SepB	-0.137	-0.044	-0.144	-0.266	-0.220	-0.280	—	—	—
SepIx	+0.002	+0.079	+0.004	+0.086	+0.087	+0.066	—	—	—
BrL	-0.238	-0.164	-0.253	-0.285	-0.237	-0.331	+0.016	+0.081	+0.155
BrB	-0.187	-0.134	-0.191	-0.123	-0.044	-0.161	+0.110	+0.164	+0.205
BrIx	-0.065	-0.030	-0.075	-0.184	-0.187	-0.192	-0.052	-0.022	+0.037
ScL	-0.242	—	-0.321	-0.255	—	-0.330	-0.055	—	+0.069
SpL	-0.261	-0.134	-0.318	+0.004	+0.167	-0.022	-0.022	+0.025	+0.068
ScTh	-0.297	-0.207	-0.359	-0.172	-0.083	-0.203	-0.020	+0.034	+0.081
LfL	+0.057	+0.224	—	+0.097	+0.237	—	-0.126	-0.133	—
LfB	-0.203	-0.121	-0.264	+0.111	+0.163	+0.074	-0.051	-0.023	+0.075
LfTh	-0.061	-0.014	-0.086	+0.103	+0.092	+0.072	-0.071	-0.051	-0.001

(a) Within the habit group there is not only the strong relationship with the grade, noted in the last section, but also different degrees of parallelism with the leaf ratio.

(b) It has already been seen that there is some connexion between spread and earliness (see § 1, FIG).

(c) Inverse tendencies appear with nearly all the measurements, though exceptions are found in the leaf group. In PMN 63, elimination of scape length, or of scape thickness which is not shown in the table, nullifies the other significant coefficients; but in PMN 44, those with sepal breadth and bract length remain unaffected, though this does not suggest any special relationship with these parts. The main interest lies in the distinctive actions of leaf and scape lengths. These two characters are positively and strongly correlated (Table XVIII), but here, in their relationship to the ratio scape spread : height, they are exerting opposing influences. Leaf length is connected with spreading of leaves (Table V) and hence indirectly with spreading of scapes (see (a) above), whereas scape length is positively correlated with erectness of scapes. Consequently elimination of scape length reveals a small positive correlation between scape spread : height and leaf length, while elimination of leaf length somewhat increases the various negative coefficients.

(d) Lastly, there is a strong positive correlation between scape spread : height and scape volume. The counteracting effect of scape length is well seen here, for after elimination the coefficients increase considerably. The long scapes tend to be

associated with narrow spread, and it must be remembered that spread affects the computation of volume to the extent of $\pi(\text{spread}/2)^2$.

(5) *Leaf spread : height, LfS : H* (Table V). This ratio bears little relationship to the other characters, and in PMN 45 there are no significant correlations at all. The other populations show variable correlations with scape habit (§§ 3 and 4) and small positive correlations with flowering grade possibly as a reflexion of these. Length of leaf is associated with spreading, especially in PMN 63. In most plants the leaves curve over, being highest in the middle, while only the young leaves have their apices in the air. It is to be expected, therefore, that long-leaved plants should tend to be more prostrate. In some types, however, the large leaves are also held rigid and raised. Leaf breadth seems to be unrelated to the ratio, unless it can be argued from the partial that, the length being equal, a narrow leaf tends to droop more than a broad one. Leaf thickness shows a positive correlation in PMN 63, but this is rendered insignificant by elimination of leaf length.

Table V. *Correlations with the ratio leaf spread : height*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		- ScL	- LfL		- ScL	- LfL		- ScL	- LfL
FIG	+0.234	+0.235	+0.059	+0.196	+0.187	+0.197	+0.160	+0.139	+0.091
HbG	-0.106	-0.148	-0.201	-0.456	-0.459	-0.444	+0.122	+0.106	+0.135
ScS : H	+0.213	+0.249	+0.208	+0.439	+0.439	+0.431	+0.048	+0.053	+0.069
ScV	+0.098	+0.022	-0.188	+0.083	+0.166	-0.010	+0.010	-0.126	-0.201
AL	+0.122	+0.104	+0.106	+0.104	+0.113	+0.120	-0.134	-0.120	-0.107
ATL	+0.059	+0.062	+0.077	+0.058	+0.055	+0.076	+0.017	+0.016	+0.005
SepL	-0.015	-0.029	-0.055	+0.129	+0.150	+0.074	-0.018	-0.078	-0.107
SepB	-0.018	-0.070	-0.063	-0.092	-0.081	-0.113	—	—	—
SepIx	+0.005	+0.042	+0.026	+0.157	+0.157	+0.127	—	—	—
BrL	+0.058	+0.018	-0.022	-0.022	-0.071	-0.075	+0.033	-0.040	-0.117
BrB	-0.037	-0.068	-0.071	+0.140	+0.169	+0.097	+0.055	+0.013	-0.030
BrIx	+0.133	+0.118	+0.078	-0.122	-0.122	-0.136	-0.012	-0.088	-0.147
ScL	+0.112	—	-0.137	-0.060	—	-0.146	+0.084	—	-0.062
SpL	+0.074	-0.005	-0.128	+0.102	+0.158	+0.063	+0.130	+0.100	+0.045
ScTh	+0.125	+0.078	-0.068	-0.043	-0.021	-0.085	+0.156	+0.142	+0.073
LfL	+0.413	+0.419	—	+0.162	+0.208	—	+0.157	+0.146	—
LfB	±0.0	-0.050	-0.249	-0.029	-0.019	-0.120	+0.170	+0.149	+0.077
LfTh	+0.285	+0.270	+0.164	-0.033	-0.036	-0.101	+0.191	+0.172	+0.127

IV. *Size of plant*

(6) *Scape volume, ScV* (Table VI). The correlations with scape volume include some of the highest values found in these studies, but here also the difference between population PMN 45 and the others becomes apparent. Although the length of the scape is of great importance in determining scape volume in all the populations, the ratio scape spread : height exerts a considerable and conflicting influence in PMN 63 and PMN 44 (see § 4(d)), whereas it has no effect in PMN 45, so that almost complete control rests with length in that population.

Next to the scape length, the strongest relationship is with length of leaf, for its elimination in partials nullifies not only direct correlations with other leaf measurements, but also some of those with sepal and bract parts. Scape length, when

eliminated in partials, swings most of the positive correlations to negative, or to zero in PMN 45, though it does not greatly affect the leaf characters. Scape thickness shows comparatively smaller direct correlations, and has much less effect in partials. Thus none of the characters, except scape spread : height, has correlations with the volume which cannot be attributed either to scape or leaf length.

V. Size and shape of organs

(7) *Anther length, AL* (Table VII). The relationship between lengths of the whole anther and of its subulate tip is positive, but significant only in PMN 63. No significant correlations are found in PMN 45, but in the other populations there are positive correlations with sepal and spike lengths. These and some other small correlations with bract or sepal parts suggest the effects of growth in the flower, but the negative correlation with leaf thickness in PMN 44 would appear to be fortuitous.

Table VII. *Correlations with anther length*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		- ScL	- LfL		- ScL	- LfL		- ScL	- LfL
ATL	+0.344	+0.354	+0.346	+0.184	+0.192	+0.177	+0.204	+0.209	+0.224
SepL	+0.281	+0.265	+0.277	+0.211	+0.186	+0.263	-0.005	+0.125	+0.102
SepB	+0.227	+0.170	+0.222	+0.111	+0.085	+0.121	—	—	—
SepIx	-0.030	+0.028	-0.027	+0.050	+0.051	+0.070	—	—	—
BrL	+0.227	+0.175	+0.220	+0.156	+0.131	+0.191	+0.009	+0.219	+0.218
BrB	+0.096	+0.053	+0.092	+0.178	+0.147	+0.151	+0.045	+0.177	+0.172
BrIx	+0.165	+0.142	+0.158	+0.007	+0.006	+0.013	-0.015	+0.149	+0.144
ScL	+0.182	—	+0.176	+0.123	—	+0.177	-0.194	—	-0.069
SpL	+0.259	+0.188	+0.259	+0.245	+0.214	+0.278	-0.088	+0.075	+0.037
ScTh	+0.161	+0.079	+0.149	+0.175	+0.139	+0.201	-0.103	+0.069	+0.029
LfL	+0.062	-0.041	—	-0.083	-0.152	—	-0.104	-0.070	—
LfB	+0.076	+0.005	+0.053	-0.103	-0.128	-0.073	-0.122	-0.008	+0.047
LfTh	+0.114	+0.081	+0.099	-0.222	-0.217	-0.207	-0.007	+0.094	+0.122

(8) *Anther tip length, ATL* (Table VIII). The length of the subulate tip of the anther is a character which has been used to distinguish between Pacific and Atlantic coast species of American sea plantains (Fernald, 1925, p. 96). Apart from the relationship with anther length, there are very few significant correlations. The effects of general plant growth fail to reach the anther tip, even in PMN 45, but floral growth affects it in PMN 44, as is shown by the correlation with sepal length, and to a less extent by that with bract length. A series of negative correlations with the leaf characters occurs in PMN 44, reaching significance with leaf thickness. These are similar to some corresponding relationships between the leaf and anther length (§ 7), and it seems probable that chance distribution accounts for this combination of anther and leaf types, as also for another inverse correlation with leaf breadth in PMN 63.

(9) *Sepal length, SepL* (Table IX). Sepal length in general shows positive correlations with the other characters, but the type of growth exemplified seems to differ in the populations. In PMN 63, the flower and its bracts form a region of

Table VIII. *Correlations with anther tip length*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		-ScL	-LfL		-ScL	-LfL		-ScL	-LfL
AL	+0.344	+0.354	+0.346	+0.184	+0.192	+0.177	+0.204	+0.209	+0.224
SepL	-0.034	-0.031	-0.032	+0.304	+0.330	+0.371	+0.181	+0.216	+0.052
SepB	+0.037	+0.050	+0.040	+0.001	+0.014	+0.012	—	—	—
SepLx	-0.039	-0.048	-0.040	+0.197	+0.197	+0.225	—	—	—
BrL	-0.053	-0.049	-0.050	+0.183	+0.202	+0.224	+0.114	+0.163	+0.087
BrB	-0.109	-0.107	-0.108	+0.061	+0.083	+0.096	+0.090	+0.105	+0.060
BrLx	+0.064	+0.068	+0.068	+0.114	+0.115	+0.122	+0.180	+0.236	+0.172
ScL	-0.021	—	-0.009	-0.053	—	-0.011	+0.002	—	-0.090
SpL	-0.093	-0.108	-0.091	+0.095	+0.144	+0.126	-0.131	-0.190	-0.220
ScTh	-0.149	-0.162	-0.154	+0.073	+0.102	+0.100	-0.072	-0.113	-0.157
LfL	-0.026	-0.017	—	-0.099	-0.084	—	+0.075	+0.117	—
LfB	-0.207	-0.217	-0.223	-0.137	-0.130	-0.103	-0.150	-0.189	-0.332
LfTh	-0.004	±0.0	+0.005	-0.234	-0.238	-0.214	+0.058	+0.064	+0.020

Table IX. *Correlations with sepal length*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		-ScL	-LfL		-ScL	-LfL		-ScL	-LfL
AL	+0.281	+0.265	+0.277	+0.211	+0.186	+0.263	-0.005	+0.125	+0.102
ATL	-0.034	-0.031	-0.032	+0.304	+0.330	+0.371	+0.181	+0.216	+0.052
SepB	+0.301	+0.279	+0.296	+0.351	+0.310	+0.336	—	—	—
SepLx	+0.348	+0.407	+0.353	+0.444	+0.462	+0.400	—	—	—
BrL	+0.512	+0.507	+0.507	+0.706	+0.686	+0.671	+0.653	+0.441	+0.509
BrB	+0.517	+0.506	+0.514	+0.461	+0.412	+0.393	+0.464	+0.242	+0.290
BrLx	+0.035	+0.017	+0.022	+0.393	+0.404	+0.395	+0.531	+0.271	+0.336
ScL	+0.120	—	+0.089	+0.263	—	+0.120	+0.553	—	+0.329
SpL	+0.137	+0.076	+0.112	+0.284	+0.177	+0.206	+0.626	+0.396	+0.483
ScTh	+0.115	+0.062	+0.087	+0.279	+0.199	+0.209	+0.646	+0.418	+0.505
LfL	+0.084	+0.024	—	+0.378	+0.304	—	+0.476	+0.087	—
LfB	+0.083	+0.039	+0.049	+0.220	+0.183	+0.051	+0.416	+0.131	+0.084
LfTh	+0.116	+0.095	+0.093	+0.114	+0.132	-0.031	+0.353	+0.132	+0.123

growth, and correlations with leaf, scape and even spike are insignificant. Growth is still strongly centred in the flower in PMN 44 but significant relationships extend to the scape and to leaf length. In PMN 45, however, the growth of the flower is merged into general growth, and strong correlations exist with the scape parts, though those with leaf dimensions are rendered insignificant in the partials.

Sepal length shows strong positive correlations with the length of the bract and also with bract breadth, but those with sepal breadth though significant are somewhat smaller. It might be expected that the parts of the flower would vary together in size, but between the length and breadth of an organ there may be some counter-acting influence, some variability in pattern, such as narrow and broad forms, which would weaken the correlation of collateral growth. The relationship with sepal index may be more conveniently considered under that heading. Sepal length has correlations of from +0.5 to zero with bract index, but this irregularity may be explained by noting the values with length and breadth of bract. When

these are equally strong as in PMN 63, the index shows no correlation, but when length is more highly correlated than breadth the index shows a positive correlation.

(10) *Sepal breadth*, **SepB** (Table X). The measurement of sepal breadth was not included in the records until after population PMN 45 had been examined. With the exception of the indices, sepal breadth shows positive correlations with all the other characters, but its relationships differ considerably from those of sepal length (§ 9). Within the flower the greatest affinity is with breadth of bract, which suggests a somewhat specific relationship, since the correlation with bract length is smaller or absent. The greater correlation with breadth than with length also determines the extent and inverse nature of the correlations with bract index, for that ratio is negatively correlated with its subsequent term, breadth. The behaviour of the bract and sepal indices can best be examined under their respective headings (§§ 14 and 11).

Table X. *Correlations with sepal breadth*

Character	PMN 63				PMN 44			
	Direct coeff.	Eliminating			Direct coeff.	Eliminating		
		-ScL	-ScTh	-LfL		-ScL	-ScTh	-LfL
AL	+0.227	+0.170	+0.181	+0.222	+0.111	+0.085	+0.053	+0.121
ATL	+0.037	+0.050	-0.105	+0.040	+0.001	+0.014	-0.026	+0.012
SepL	+0.301	+0.279	+0.281	+0.296	+0.351	+0.310	+0.282	+0.336
SepIx	-0.772	-0.744	-0.743	-0.772	-0.652	-0.668	-0.659	-0.697
BrL	+0.150	-0.001	+0.040	+0.135	+0.260	+0.215	+0.182	+0.238
BrB	+0.513	+0.464	+0.466	+0.510	+0.430	+0.386	+0.362	+0.419
BrIx	-0.317	-0.420	-0.384	-0.337	-0.087	-0.093	-0.114	-0.097
ScL	+0.407	—	+0.260	+0.422	+0.231	—	+0.109	+0.203
SpL	+0.280	+0.001	-0.010	+0.266	+0.167	+0.056	-0.025	+0.143
ScTh	+0.390	+0.230	—	+0.390	+0.352	+0.293	—	+0.337
LfL	+0.095	-0.155	-0.091	—	+0.113	+0.015	+0.031	—
LfB	+0.374	+0.255	+0.167	+0.376	+0.260	+0.229	+0.130	+0.236
LfTh	+0.042	-0.043	-0.045	+0.009	+0.084	+0.099	+0.022	+0.046

Outside the flower, sepal breadth has positive correlations with scape thickness, which are unimpaired by elimination of scape length. It also shows a good correlation with leaf breadth, though this is reduced to insignificance by elimination of scape thickness. Thus there are signs of a common growth in breadth, cutting across the groups of characters, and centred in scape thickness. On the other hand, there is a good correlation with scape length in PMN 63, so that the growth influences are at least partly of a general nature.

(11) *Sepal index (length : breadth)*, **SepIx** (Tables XI and XI(a)). Ratios like sepal index are convenient "characters" for taxonomic description, and it is therefore desirable to note the extent of their dependence on other characters, though this is in the nature of pseudo-correlation due to correlations with one or both of the ratio terms. Table XI, in which the direct and partial correlations are set out in the usual form, would convey little unless reference were also made to the sepal length and sepal breadth tables, so Table XI(a) has been compiled to compare the direct coefficients of the ratio and its terms.

Table XI. *Correlations with sepal index*

Character	PMN 63				PMN 44			
	Direct coeff.	Eliminating			Direct coeff.	Eliminating		
		-ScL	-ScTh	-LfL		-ScL	-ScTh	-LfL
AL	-0.030	+0.028	+0.022	-0.027	+0.050	+0.051	+0.070	+0.070
ATL	-0.039	-0.048	-0.091	-0.040	+0.197	+0.197	+0.207	+0.225
SepL	+0.348	+0.407	+0.406	+0.353	+0.444	+0.462	+0.496	+0.400
SepB	-0.772	-0.744	-0.743	-0.772	-0.652	-0.652	-0.659	-0.697
BrL	+0.226	+0.385	+0.350	+0.239	+0.306	+0.317	+0.350	+0.256
BrB	-0.157	-0.085	-0.086	-0.154	-0.041	-0.042	-0.009	-0.115
BrIx	+0.364	+0.438	+0.415	+0.376	+0.404	+0.404	+0.413	+0.398
ScL	-0.308	—	-0.180	-0.336	-0.005	—	+0.040	-0.113
SpL	-0.168	+0.064	+0.095	-0.165	+0.071	+0.086	+0.152	+0.014
ScTh	-0.313	-0.188	—	-0.326	-0.108	-0.115	—	-0.169
LfL	-0.045	+0.146	+0.108	—	+0.218	+0.244	+0.253	—
LfB	-0.301	-0.205	-0.132	-0.319	-0.054	-0.054	-0.009	-0.182
LfTh	+0.036	+0.104	+0.110	+0.055	+0.035	+0.035	+0.056	-0.050

Table XI(a). *Direct correlations of the sepal characters*

Character	PMN 63			PMN 44		
	SepIx			SepIx		
	+0.348	-0.772		+0.444	-0.652	
	SepL	+0.301	SepB	SepL	+0.351	SepB
	SepL	SepB	SepIx	SepL	SepB	SepIx
AL	+0.281	+0.227	-0.030	+0.221	+0.111	+0.050
ATL	-0.034	+0.037	-0.039	+0.304	+0.001	+0.197
BrL	+0.512	+0.150	+0.226	+0.706	+0.260	+0.306
BrB	+0.517	+0.513	-0.157	+0.461	+0.430	-0.041
BrIx	+0.035	-0.317	+0.364	+0.393	-0.087	+0.404
ScL	+0.120	+0.407	-0.308	+0.263	+0.231	-0.005
SpL	+0.137	+0.280	-0.168	+0.284	+0.167	+0.071
ScTh	+0.115	+0.390	-0.313	+0.279	+0.352	-0.108
LfL	+0.084	+0.095	-0.045	+0.378	+0.113	+0.218
LfB	+0.083	+0.374	-0.301	+0.220	+0.260	-0.054
LfTh	+0.116	+0.042	+0.036	+0.114	+0.084	+0.035

The relationships *within* the sepal characters are alike in both populations; a small positive correlation between length and breadth; a slightly higher positive between length and index; and a high negative between index and breadth. It may be concluded that the size of both dimensions affects the value of the index, but that breadth, in the inverse direction, is the more important.

The nature and intensity of the correlations formed by the index with various size characters appear to be based on two considerations, though these may be related. First, if length has a greater correlation than breadth the index will have a correlation of the same sign as that of length; and conversely, if breadth has the greater correlation the index will give one with changed sign because breadth is inversely correlated with index. The second consideration seems to be a bias in favour of breadth, which may be attributed to the greater activity of breadth in forming the ratio and/or to its greater variability (which may be seen from the

data in Table XXII). This bias can be discerned by comparing the coefficients of length, breadth and index in Table XI(a). For example the relationships of anther tip length in PMN 44 give (1) with length $+0.304$, (2) with breadth $+0.001$, making a difference of 0.303 in favour of length, but (3) with index the correlation is only $+0.197$. Contrast with this the correlations of scape thickness in PMN 63, viz. (1) with length $+0.115$, (2) with breadth $+0.390$, a difference of 0.275 in favour of breadth, but the value with (3) index is -0.313 , which is thus greater than the difference. The subject will be pursued in § 14 for bracts, and further considered in the Discussion.

(12) *Bract length, BrL* (Table XII). Like sepal length (§ 9), bract length gives an array of positive correlations with the other characters. The chief difference may be found in PMN 63, in which the relationships of the sepal were confined to the flower, whereas those of the bract extend to the scape parts and to leaf breadth. In general, however, PMN 63 and PMN 44 again suggest a growth centred in the flower, while PMN 45 shows a superimposition of general plant growth. There is a stronger affinity between length and breadth of bract than was found in the corresponding sepal dimensions, and whereas sepal length was strongly correlated with bract breadth ($+0.46$ to $+0.52$), the relationship between bract length and sepal breadth is much less certain. Sepal index, as was seen in the last section, has a strong positive correlation with bract index, and probably on that account it also has a positive and significant relationship with bract length. The relationships with leaf parts are very vague, and will be reviewed in later sections.

Table XII. *Correlations with bract length*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		-ScL	-LfL		-ScL	-LfL		-ScL	-LfL
AL	$+0.227$	$+0.175$	$+0.220$	$+0.156$	$+0.131$	$+0.191$	$+0.009$	$+0.219$	$+0.218$
ATL	-0.053	-0.049	-0.050	$+0.183$	$+0.202$	$+0.224$	$+0.114$	$+0.163$	$+0.087$
SepL	$+0.512$	$+0.507$	$+0.507$	$+0.706$	$+0.686$	$+0.671$	$+0.653$	$+0.441$	$+0.509$
SepB	$+0.150$	-0.001	$+0.136$	$+0.260$	$+0.215$	$+0.238$	—	—	—
SepIx	$+0.226$	$+0.385$	$+0.239$	$+0.306$	$+0.317$	$+0.258$	—	—	—
BrB	$+0.531$	$+0.486$	$+0.529$	$+0.539$	$+0.500$	$+0.493$	$+0.702$	$+0.545$	$+0.557$
BrIx	$+0.515$	$+0.499$	$+0.501$	$+0.589$	$+0.604$	$+0.596$	$+0.757$	$+0.545$	$+0.556$
ScL	$+0.370$	—	$+0.324$	$+0.246$	—	$+0.136$	$+0.722$	—	$+0.369$
SpL	$+0.328$	$+0.109$	$+0.278$	$+0.292$	$+0.198$	$+0.237$	$+0.701$	$+0.382$	$+0.483$
ScTh	$+0.293$	$+0.128$	$+0.238$	$+0.273$	$+0.198$	$+0.217$	$+0.688$	$+0.313$	$+0.424$
LfL	$+0.189$	-0.009	—	$+0.301$	$+0.222$	—	$+0.720$	$+0.364$	—
LfB	$+0.256$	$+0.130$	$+0.192$	$+0.079$	$+0.038$	-0.074	$+0.452$	$+0.040$	-0.246
LfTh	$+0.100$	$+0.030$	$+0.037$	$+0.199$	$+0.219$	$+0.099$	$+0.517$	$+0.300$	$+0.208$

(13) *Bract breadth, BrB* (Table XIII). The tendency for collateral growth in breadth, which was suspected in sepal breadth (§ 10), is not so apparent here. Bract breadth is strongly correlated with sepal breadth, but equally with sepal length, which incidentally explains the absence of correlation with sepal index. The relationship with scape thickness is fairly good, but that with scape length is stronger except in PMN 45. Lastly, the correlation with leaf breadth is outmatched

by that with leaf length except in PMN 63. The results therefore suggest the effects of undifferentiated growth. Although the coefficients of variation for the indices of sepal and bract are similar (Table XXII), it would seem that increase in length and breadth is due more frequently to a common cause in the bract than in the sepal. The populations differ, however, and PMN 45 as usual has a series of high correlations, while these are concentrated in the flower in the other two.

Table XIII. *Correlations with bract breadth*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		-ScL	-LfL		-ScL	-LfL		-ScL	-LfL
AL	+0.096	+0.053	+0.092	+0.178	+0.147	+0.151	+0.045	+0.177	+0.172
ATL	-0.109	-0.107	-0.108	+0.061	+0.083	+0.096	+0.090	+0.105	+0.060
SepL	+0.517	+0.506	+0.514	+0.461	+0.412	+0.393	+0.464	+0.242	+0.290
SepB	+0.513	+0.464	+0.510	+0.430	+0.386	+0.419	—	—	—
SepLx	-0.157	-0.085	-0.154	-0.041	-0.042	-0.115	—	—	—
BrL	+0.531	+0.486	+0.529	+0.539	+0.500	+0.493	+0.702	+0.545	+0.557
BrLx	-0.441	-0.502	-0.458	-0.309	-0.331	-0.348	+0.153	-0.299	-0.267
ScL	+0.253	—	+0.257	+0.326	—	+0.227	+0.529	—	+0.238
SpL	+0.198	+0.034	+0.187	+0.270	+0.123	+0.207	+0.647	+0.453	+0.493
ScTh	+0.249	+0.142	+0.245	+0.306	+0.206	+0.252	+0.616	+0.388	+0.434
LfL	+0.067	-0.081	—	+0.302	+0.189	—	+0.515	+0.193	—
LfB	+0.367	+0.301	+0.382	+0.248	+0.205	+0.125	+0.403	+0.129	+0.005
LfTh	+0.145	+0.101	+0.130	+0.206	+0.237	+0.106	+0.381	+0.182	+0.136

(14) *Bract index (length : breadth), BrLx* (Tables XIV and XIV(a)). Populations PMN 63 and PMN 44 are alike in the correlations *within* their bract groups (Table XIV(a)), but PMN 45 differs at least in degree. These three relationships have been calculated in ten other samples, and a considerable range of coefficient values has been found, in which the correlation *Ix-B* diminishes as those of *L-B* and *L-Ix* increase. The condition of PMN 45 is extreme among these, and it is the only sample in which the correlation *Ix-B* has a positive sign. It should be mentioned, also, that the coefficients of *L-B* and *L-Ix* are not necessarily so similar as the present examples suggest. When examining sepal index in § 11, it was noted that the correlation between index and any character was partly determined by the greater degree of correlation shown by length, or breadth, with that character, but there also appeared to be a bias in favour of breadth in the sepals. Here, in the bract group, length is more active than breadth and the variability of length is the greater, especially in PMN 45 (see Table XXII). It is of interest, therefore, to distinguish a bias in favour of length, even in PMN 63, where the opposing influences are almost equal. In PMN 45 breadth exerts no opposing influence; and in consequence the index has all the appearance of a character strongly affected by growth. The indices of PMN 63 and PMN 44, on the other hand, are practically unaffected by growth, because (a) the terms length and breadth have counteracting influences, (b) there is little difference in the correlations formed by the terms with other characters, and (c) there is little bias. A ratio, therefore, can afford a useful diagnostic character, relatively unaffected by growth; but, under conditions which

appear to have been infrequent in this experimental garden, even a ratio may increase or diminish according to the size of the plant.

Table XIV. *Correlations with bract index*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		-ScL	-LfL		-ScL	-LfL		-ScL	-LfL
AL	+0.165	+0.142	+0.158	+0.007	+0.006	+0.013	-0.015	+0.149	+0.144
ATL	+0.064	+0.068	+0.068	+0.114	+0.115	+0.122	+0.180	+0.236	+0.172
SepL	+0.035	+0.017	+0.022	+0.393	+0.404	+0.395	+0.531	+0.271	+0.336
SepB	-0.317	-0.420	-0.337	-0.087	-0.093	-0.097	—	—	—
SepIx	+0.364	+0.438	+0.376	+0.404	+0.404	+0.398	—	—	—
BrL	+0.515	+0.499	+0.501	+0.589	+0.604	+0.596	+0.757	+0.545	+0.556
BrB	-0.441	-0.502	-0.458	-0.309	-0.331	-0.348	+0.153	-0.299	-0.267
ScL	+0.152	—	+0.086	+0.012	—	+0.022	+0.652	—	+0.319
SpL	+0.160	+0.077	+0.105	+0.120	+0.134	+0.105	+0.553	+0.163	+0.275
ScTh	+0.090	+0.013	+0.026	+0.055	+0.055	+0.039	+0.517	+0.047	+0.181
LfL	+0.152	+0.085	—	+0.074	+0.076	—	+0.638	+0.274	—
LfB	-0.090	-0.165	-0.188	-0.113	-0.117	-0.168	+0.336	-0.085	-0.330
LfTh	+0.010	-0.029	-0.047	+0.015	+0.016	-0.013	+0.402	+0.151	+0.078

Table XIV(a). *Direct correlations of the bract characters*

Character	PMN 63			PMN 44			PMN 45		
	BrIx			BrIx			BrIx		
	+0.515	-0.441		+0.589	-0.309		+0.757	+0.153	
	BrL	+0.531	BrB	BrL	+0.539	BrB	BrL	+0.702	BrB
	BrL	BrB	BrIx	BrL	BrB	BrIx	BrL	BrB	BrIx
AL	+0.277	+0.096	+0.165	+0.156	+0.178	+0.007	+0.009	+0.045	-0.015
ATL	-0.053	-0.109	+0.064	+0.183	+0.061	+0.114	+0.114	+0.090	+0.180
SepL	+0.512	+0.517	+0.035	+0.706	+0.461	+0.393	+0.653	+0.464	+0.531
SepB	+0.150	+0.513	-0.317	+0.260	+0.430	-0.087	—	—	—
SepIx	+0.226	-0.157	+0.364	+0.306	-0.041	+0.404	—	—	—
ScL	+0.370	+0.253	+0.152	+0.246	+0.326	+0.012	+0.722	+0.529	+0.652
SpL	+0.328	+0.198	+0.160	+0.292	+0.270	+0.120	+0.701	+0.647	+0.553
ScTh	+0.293	+0.249	+0.090	+0.273	+0.306	+0.055	+0.688	+0.616	+0.517
LfL	+0.189	+0.067	+0.152	+0.301	+0.302	+0.074	+0.720	+0.515	+0.638
LfB	+0.256	+0.367	-0.090	+0.079	+0.248	-0.113	+0.452	+0.403	+0.336
LfTh	+0.110	+0.145	+0.010	+0.199	+0.206	+0.015	+0.517	+0.381	+0.402

(15) *Scape length, ScL* (Table XV). In choosing a single dimension like scape length to represent growth, as has been done for the partials of this paper, it should be examined to discover whether there are any peculiarities of behaviour which might impair its usefulness. First, the factors controlling scape length might be so specific that they exerted no other influence. Obviously this is not the case, for length and thickness of scape are controlled by common causes to a considerable though varying extent; while that section of the scape axis denoted as spike length varies proportionately to the whole. Secondly, the scape might be controlled independently of other organs, but there is strong correlation with leaf length and variable correlation with floral and bract parts, except the anthers which are independent. Thirdly, these relationships with other organs might be of some special

character, e.g. parallel variation in length. But the relationships with bracts and sepals are somewhat haphazard, sometimes length and sometimes breadth being the more highly correlated. The effects of eliminating scape thickness from the partials of these shows that general size rather than length lies behind the relationships. With the leaf, the relationship does appear to be one between lengths, and as a corollary it will be found in § 19 that leaf breadth has affinities to thickness of other parts. It should be noted, however, that leaf length is by far the most active character in determining leaf size. It is much more variable than the others, while the mean values (Table XXII) show it to be at least twenty-five times as great as breadth and one hundred times the size of thickness.

Table XV. *Correlations with scape length*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		-ScTh	-LfL		-ScTh	-LfL		-ScTh	-LfL
AL	+0.182	+0.117	+0.176	+0.123	+0.061	+0.177	-0.194	-0.178	-0.069
ATL	-0.021	+0.067	-0.009	-0.053	-0.030	-0.011	+0.002	+0.087	-0.090
SepL	+0.120	+0.071	+0.089	+0.263	+0.175	+0.120	+0.553	+0.127	+0.329
SepB	+0.407	+0.260	+0.422	+0.027	-0.127	-0.024	—	—	—
SepLx	-0.308	-0.180	-0.336	-0.005	+0.040	-0.113	—	—	—
BrL	+0.370	+0.266	+0.324	+0.246	+0.158	+0.136	+0.722	+0.423	+0.369
BrB	+0.253	+0.150	+0.257	+0.326	+0.236	+0.227	+0.529	+0.122	+0.238
BrLx	+0.152	+0.124	+0.086	+0.012	-0.010	-0.022	+0.652	+0.465	+0.319
SpL	+0.687	+0.528	+0.599	+0.524	+0.407	+0.471	+0.716	+0.317	+0.494
ScTh	+0.518	—	+0.376	+0.388	—	+0.325	+0.758	—	+0.535
LfL	+0.528	+0.392	—	+0.431	+0.378	—	+0.780	+0.586	—
LfB	+0.393	+0.077	+0.186	+0.174	+0.011	-0.036	+0.595	+0.200	-0.027
LfTh	+0.197	+0.105	+0.014	-0.053	-0.136	-0.255	+0.462	-0.002	+0.061

On the whole scape length may be regarded as a fairly efficient indicator of growth, though incomplete because it cannot record any independent manifestations of thickness. The special relationships of scape length with scape habit, and its bearing on the calculation of scape volume have been discussed in §§ 3 and 6.

(16) *Spike length, SpL* (Table XVI). Spike length is highly correlated both with scape length, of which it forms a part, and with scape thickness. The coefficients of the latter relationships are indeed slightly stronger in each population, so that not only is there harmony between the lengths of the scape parts, but the spike is also borne upon a stem of appropriate strength. There is no true correlation with the leaf characters since the partials are all insignificant except leaf breadth in PMN 63. Positive correlations of different values are found between spike length and the various floral characters. Those of PMN 45 are high in accordance with the powerful general growth found in that population. In PMN 63 and PMN 44, however, they are smaller and vary, while the indices of bract and sepal give insignificant values. It is of interest to examine whether spike length has a closer connexion with these parts than has scape length. If Tables XV and XVI are compared, it will be seen that spike length shows significant correlations with anther length, whereas scape length has only insignificant tendencies. With the bract and

sepal measurements, however, sometimes scape length and sometimes spike length shows the stronger correlation. It was also found in § 2, that spike length has greater inverse correlations with spike density than scape length. On the whole, therefore, it cannot be claimed that spike length bears any special relationship to the growth of the flower which is not at least shared by scape length.

Table XVI. *Correlations with spike length*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		-ScL	-LfL		-ScL	-LfL		-ScL	-LfL
AL	+0.259	+0.188	+0.259	+0.245	+0.214	+0.278	-0.088	+0.075	+0.037
ATL	-0.093	-0.108	-0.091	+0.095	+0.144	+0.126	-0.131	-0.190	-0.220
SepL	+0.137	+0.076	+0.112	+0.284	+0.177	+0.206	+0.626	+0.396	+0.483
SepB	+0.280	+0.001	+0.266	+0.167	+0.056	+0.143	—	—	—
SepIx	-0.168	+0.064	-0.165	+0.071	+0.086	+0.014	—	—	—
BrL	+0.328	+0.109	+0.278	+0.292	+0.198	+0.237	+0.701	+0.382	+0.483
BrB	+0.198	+0.034	+0.187	+0.270	+0.123	+0.207	+0.647	+0.453	+0.493
BrIx	+0.160	+0.077	+0.105	+0.120	+0.134	+0.105	+0.553	+0.163	+0.275
ScL	+0.687	—	+0.599	+0.524	—	+0.471	+0.716	—	+0.494
ScTh	+0.733	+0.607	+0.671	+0.530	+0.416	+0.498	+0.770	+0.500	+0.627
LfL	+0.434	+0.115	—	+0.264	+0.050	—	+0.601	+0.097	—
LfB	+0.479	+0.314	+0.343	+0.063	-0.034	-0.072	+0.491	+0.117	+0.049
LfTh	+0.266	+0.183	+0.134	+0.038	+0.078	-0.067	+0.431	+0.162	+0.150

(17) *Scape thickness, ScTh* (Table XVII). Scape thickness shows positive correlations with all the other measurements except those of the anther. Once again the series in PMN 45 has very high values, while those of the other populations vary in magnitude. The strong relationship with spike length is not greatly affected in the partials, and is higher than that with scape length. Correlations with leaf length are strongly reduced or nullified by elimination of scape length, but the high correlations with leaf breadth remain fully significant, indicating a more real relationship. Scape thickness also shows stronger correlations with sepal breadth than with sepal length, but no differentiation between length and breadth of bract, and no true connexion with leaf thickness, except in PMN 45. There seems therefore to be some differentiation of growth in thickness or breadth in contrast to length, though some of the links of the series are weak or missing.

Scape thickness has been used as a second indicator of general growth in the scape region. It has been employed in partial correlations to determine whether growth in thickness can be distinguished from growth in length. The data have had to be omitted from most of the tables, but are presented in seven of them. It can be assumed in the others that they showed effects similar to, or slightly less pronounced than, those of the scape length partials. The most marked divergences are best seen in Tables XIX and VI, for thickness is of chief importance in the leaf breadth correlations, while in those of scape volume length is an important component and scape thickness has only an indirect bearing so that it affects the coefficients to a smaller extent.

Table XVII. *Correlations with scape thickness*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		-ScL	-LfL		-ScL	-LfL		-ScL	-LfL
AL	+0.161	+0.079	+0.149	+0.175	+0.139	+0.201	-0.103	+0.069	+0.029
ATL	-0.149	-0.162	-0.154	+0.073	+0.102	+0.100	-0.072	-0.113	-0.157
SepL	+0.115	+0.062	+0.087	+0.279	+0.199	+0.209	+0.646	+0.418	+0.505
SepB	+0.390	+0.230	+0.390	+0.352	+0.293	+0.337	—	—	—
SepLx	-0.313	-0.188	-0.326	-0.108	-0.115	-0.169	—	—	—
BrL	+0.293	+0.128	+0.238	+0.273	+0.198	+0.217	+0.688	+0.313	+0.424
BrB	+0.249	+0.142	+0.245	+0.306	+0.206	+0.252	+0.616	+0.388	+0.434
BrLx	+0.090	+0.013	+0.026	+0.055	+0.055	+0.039	+0.517	+0.047	+0.181
ScL	+0.518	—	+0.376	+0.388	—	+0.325	+0.758	—	+0.535
SpL	+0.733	+0.607	+0.671	+0.530	+0.416	+0.498	+0.770	+0.500	+0.627
LfL	+0.437	+0.225	—	+0.240	+0.087	—	+0.643	+0.126	—
LfB	+0.662	+0.584	+0.573	+0.424	+0.393	+0.364	+0.655	+0.389	+0.324
LfTh	+0.212	+0.131	+0.069	+0.180	+0.218	+0.101	+0.611	+0.451	+0.401

(18) *Leaf length, LfL* (Table XVIII). The effects of leaf length in partials can be contrasted with those of the scape characters. Besides indicating the size of a plant by its leaves, this represents a period of vegetative activity before flowering has commenced. It may be mentioned that the first formed leaves continue to lengthen until July. Measurement on a definite date, 15 May, not only finds the plants of a population at different stages of development, but may also disclose differences between populations. Certainly the correlations with leaf length are not alike in these populations, for PMN 45 shows the same display of high positive values which has characterized the foregoing tables; PMN 44 tends to approach this condition with lower values; while PMN 63 has correlations only amongst the leaf and scape characters, and no significant relationships with bract, sepal or anther.

The correlations between length and breadth of leaf are moderately high in PMN 63 and PMN 44, and very high in PMN 45; while length and thickness

Table XVIII. *Correlations with leaf length*

Character	PMN 63			PMN 44			PMN 45		
	Direct coeff.	Eliminating		Direct coeff.	Eliminating		Direct coeff.	Eliminating	
		-ScL	-ScTh		-ScL	-ScTh		-ScL	-ScTh
AL	+0.062	-0.041	-0.009	-0.083	-0.152	-0.131	-0.194	-0.070	-0.168
ATL	-0.026	-0.017	+0.045	-0.099	-0.084	-0.120	+0.075	+0.117	+0.159
SepL	+0.084	+0.024	+0.038	+0.378	+0.304	+0.334	+0.476	+0.087	+0.105
SepB	+0.095	-0.155	-0.091	+0.113	+0.015	+0.031	—	—	—
SepLx	-0.045	+0.146	+0.108	+0.218	+0.244	+0.253	—	—	—
BrL	+0.189	-0.009	+0.070	+0.301	+0.222	+0.252	+0.720	+0.364	+0.500
BrB	+0.067	-0.081	-0.048	+0.302	+0.189	+0.247	+0.515	+0.193	+0.198
BrLx	+0.152	+0.085	+0.126	+0.074	+0.076	+0.062	+0.638	+0.274	+0.467
ScL	+0.528	—	+0.392	+0.431	—	+0.378	+0.780	—	+0.586
SpL	+0.434	+0.115	+0.186	+0.264	+0.050	+0.166	+0.601	+0.097	+0.216
ScTh	+0.437	+0.225	—	+0.240	+0.087	—	+0.643	+0.126	—
LfB	+0.481	+0.346	+0.296	+0.471	+0.445	+0.419	+0.776	+0.621	+0.614
LfTh	+0.352	+0.297	+0.295	+0.371	+0.437	+0.343	+0.551	+0.344	+0.262

correlations are lower in each case but fully significant. As might be expected, the scape measurements have practically no effect in the partials of these. The chief relationship in the scape region is that with scape length. The correlations with spike length and scape thickness are nullified or strongly reduced by eliminating scape length. In PMN 44, the series of correlations between leaf length and the bract and sepal measurements are reduced but not rendered insignificant by eliminating scape length. It may be considered that leaf length comes under the influence of general growth completely in PMN 45, and to a less extent in PMN 44, but that in PMN 63 the influence is restricted mainly to lengths of leaf and scape, the thickness of the latter being only indirectly concerned.

(19) *Leaf breadth, LfB* (Table XIX). Breadth of leaf is a very conspicuous character of the plant, but the correlations show that its variations may be accompanied by parallel growth in both leaf length and scape thickness. The latter relationship suggests another aspect of collateral growth in breadth (see §§ 10 and 13). Scape thickness is the pivot of these tendencies, and here at least scape length has no importance. Such correlations as exist between leaf breadth and leaf thickness are shown to be almost entirely due to common relationships with leaf length. In PMN 45 there are again high positive correlations which are reduced to insignificance in the partials.

(20) *Leaf thickness, LfTh* (Table XX). Leaf thickness is a variable and uncertain character, partly because it is measured on the turgid tissues of the soft midrib, which contract appreciably if wilted, and which may be affected by the water supply previously available to that particular leaf. The only consistent relationship is with leaf length. In PMN 45 all the other correlations except that with scape thickness are insignificant in the partials. In PMN 44, some relationship exists with leaf breadth, and there are small inverse correlations with the anther which seem to be accidental.

SIMILARITY OF SAMPLES

The foregoing tables have shown series of correlation values, which do not occur haphazardly, but for the most part in orderly sequences graded in strength according to the positions of the characters involved. Nevertheless the three population samples are not alike, and the question arises as to the causes of their dissimilarity. They were chosen more to represent the collection than to determine the exact conditions in one small section. The differences may therefore be either inherent in the populations or the result of environmental conditions. There are no striking differences between the behaviour of PMN 63 and that of PMN 44, yet these are samples of tetraploid *Plantago serpentina* and diploid *P. maritima* respectively, though both were from inland habitats whereas PMN 45 was coastal.

The results obtained in PMN 45 seem to have been less usual, under the experimental conditions, than those of the other two. There is reason to believe that the plot of PMN 45 contained an area of distinctly lower soil fertility and that the plants therein were relatively under-developed. This was suspected from examination of the records, and to some extent confirmed by testing the normality of

Table XIX. Correlations with leaf breadth

Character	PMN 63				PMN 44				PMN 45			
	Direct coeff.	Eliminating			Direct coeff.	Eliminating			Direct coeff.	Eliminating		
		-ScL	-ScTh	-LfL		-ScL	-ScTh	-LfL		-ScL	-ScTh	-LfL
AL	+0.076	+0.005	-0.041	+0.053	-0.103	-0.128	-0.199	-0.073	-0.122	-0.008	-0.073	+0.047
ATL	-0.207	-0.217	-0.146	-0.223	-0.137	-0.130	-0.186	-0.103	-0.150	-0.189	-0.137	-0.332
SepL	+0.083	+0.039	+0.009	+0.049	+0.220	+0.183	+0.117	+0.051	+0.416	+0.131	-0.012	+0.084
SepB	+0.374	+0.255	+0.167	+0.376	+0.260	+0.229	+0.130	+0.236	—	—	—	—
Sepix	-0.301	-0.205	-0.132	-0.319	-0.054	-0.054	-0.009	-0.182	—	—	—	—
BrL	+0.256	+0.130	+0.087	+0.192	+0.079	+0.038	-0.042	-0.074	+0.452	+0.040	+0.001	-0.246
BrB	+0.367	+0.301	+0.278	+0.382	+0.248	+0.205	+0.137	+0.125	+0.403	+0.129	-0.001	+0.005
BrLx	-0.090	-0.165	-0.201	-0.188	-0.113	-0.117	+0.011	-0.168	+0.336	-0.085	-0.005	-0.330
SeL	+0.393	—	+0.077	+0.186	-0.174	—	—	-0.036	+0.595	+0.117	+0.200	-0.027
SpL	+0.479	+0.314	-0.012	+0.343	+0.063	-0.034	-0.211	-0.072	+0.491	+0.389	-0.028	+0.049
ScTh	+0.662	+0.584	—	+0.573	+0.424	+0.393	—	+0.364	+0.655	+0.621	+0.614	—
LfL	+0.481	+0.346	+0.296	—	+0.471	+0.445	+0.419	—	+0.776	+0.621	+0.614	—
LfTh	+0.249	+0.131	+0.152	+0.097	+0.347	+0.362	+0.304	+0.210	+0.497	+0.312	+0.162	+0.131

Table XX. Correlations with leaf thickness

Character	PMN 63				PMN 44				PMN 45			
	Direct coeff.	Eliminating			Direct coeff.	Eliminating			Direct coeff.	Eliminating		
		-ScL	-ScTh	-LfL		-ScL	-ScTh	-LfL		-ScL	-ScTh	-LfL
AL	+0.114	+0.081	+0.083	+0.099	-0.222	-0.217	-0.262	-0.207	-0.007	+0.004	+0.070	+0.122
ATL	-0.004	±0.0	+0.029	+0.005	-0.234	-0.238	-0.252	-0.214	+0.058	+0.064	+0.129	+0.020
SepL	+0.116	+0.095	+0.094	+0.093	+0.114	+0.132	+0.067	-0.031	+0.058	+0.132	-0.069	+0.123
SepB	+0.042	-0.043	-0.045	+0.009	+0.084	+0.099	+0.022	+0.046	—	—	—	—
SepLx	+0.036	+0.104	+0.110	+0.055	+0.035	+0.035	+0.056	-0.050	—	—	—	—
BrL	+0.100	+0.030	+0.041	+0.037	+0.199	-0.219	+0.158	+0.099	+0.517	+0.300	+0.169	+0.208
BrB	+0.145	+0.101	+0.098	+0.130	+0.206	-0.237	+0.161	+0.106	+0.381	+0.182	+0.008	+0.136
BrLx	+0.010	-0.029	-0.009	-0.047	+0.015	+0.016	+0.006	-0.013	+0.402	+0.152	+0.128	+0.078
ScL	+0.197	—	+0.105	+0.014	-0.053	—	-0.136	-0.255	+0.462	+0.162	-0.002	+0.061
Spl	+0.266	+0.183	+0.166	+0.134	+0.038	+0.078	-0.008	-0.067	+0.431	+0.162	-0.078	+0.150
ScTh	+0.212	+0.131	—	+0.069	+0.180	-0.218	—	+0.101	+0.611	+0.451	—	+0.401
LfL	+0.352	+0.297	+0.295	+0.097	+0.371	+0.437	+0.343	—	+0.551	+0.344	+0.262	+0.131
LfB	+0.249	+0.131	+0.152	+0.097	+0.347	+0.362	+0.304	+0.210	+0.497	+0.312	+0.162	+0.131

distribution curves for various characters. It has not been possible to grow another sample of PMN 45, but a number of other populations have been roughly examined by calculating a few critical correlations, and none seemed likely to repeat the performance. Samples of PMN 44, however, have been included in the trials for several years. Further studies are being undertaken to compare the behaviour of certain correlations in these, and it is already obvious that there may be considerable variation. For example, the relationship of scape length with mature leaf length varies from $+0.277$ to $+0.672$. In Table XXI certain groups of direct correlations from the 1933 and 1935 samples are compared. The 1935 data were employed in the first paper (Gregor *et al.* 1936) and are printed below and to the left of each diagonal; while the corresponding figures of the 1933 sample—the subject of this paper—are given above and on the right. Except for a few discrepancies the two sets agree fairly closely. There was, however, a higher degree of association within the scape group in 1935.

Table XXI. Comparison of years (PMN 44)

Character	LfL	1933 Sample		Character	SpL	1933 Sample	
		LfB	LfTh			ScL	ScTh
LfL	—	$+0.471$	$+0.371$	SpL	—	$+0.524$	$+0.530$
LfB	$+0.456$	—	$+0.347$	ScL	$+0.734$	—	$+0.388$
LfTh	$+0.218$	$+0.196$	—	ScTh	$+0.719$	$+0.596$	—
1935 Sample				1935 Sample			

Character	SpD	1933 Sample					
		SepL	SepB	SepIx	BrL	BrB	BrIx
SpD	—	-0.276	-0.110	-0.102	-0.253	-0.131	-0.198
SepL	-0.265	—	$+0.351$	$+0.444$	$+0.706$	$+0.461$	$+0.393$
SepB	-0.169	$+0.318$	—	-0.652	$+0.260$	$+0.430$	-0.087
SepIx	-0.043	$+0.442$	-0.671	—	$+0.306$	-0.041	$+0.404$
BrL	-0.286	$+0.655$	$+0.304$	$+0.228$	—	$+0.539$	$+0.589$
BrB	-0.331	$+0.463$	$+0.488$	-0.096	$+0.628$	—	-0.309
BrIx	-0.020	$+0.383$	-0.094	$+0.388$	$+0.649$	-0.167	—
1935 Sample							

It seems probable that the less uniform the conditions of growth the higher the values obtained for correlations due to collateral growth, but so far it has not been possible to distinguish heredity from other causes of non-uniformity, such as local environment and the condition of the seed. That hereditary variation exists, however, is apparent from the large and significant differences in mean values which occur between the characters of various populations (Gregor *et al.* 1936; Gregor, 1938).

DISCUSSION

Alkins (1920-1) obtained positive values of over $+0.9$ when correlating measurements of length, width and thickness in small molluscs. He also found a correlation of about $+0.5$ between the ratios width: length and thickness: length. Such

material may be unusually favourable for the expression of monotypic uniformity, but the point of interest is that practically identical results were obtained in several local races of different *Sphaeria* species. For various size measurements on the crab, *Carcinus moenas*, Weldon (1893) obtained correlations ranging from +0.8 to -0.26, which are more comparable with those found in the present material, but here again two local races, at Plymouth and Naples, showed coefficients which corresponded without significant differences. Weldon therefore concluded that Galton's function (an estimate of r) has the same value in all local races. To some extent this tendency for local races to show similar values of correlation might be expected in plant species also; but a plant is viable under such wide ranges of variation and modification, that differences in the strength of correlation are theoretically possible. Large variations, e.g. giant and dwarf forms, might strongly affect the plant proportions, while genetical linkage might set up special forms of correlation. Hence it may be argued that smaller variations would also affect the degree of correlation. Again, as an extreme example of modification it might be imagined that plants which normally had a positive correlation between lengths of stem and leaf would show an inverse tendency if modified by various intensities of etiolation. Even with larger numbers of observations precision such as can be obtained on the hard parts of shellfish cannot be expected from the indefinite growth of the plantain plant.

When Sumner (1923) investigated correlations in a local race of the deer-mouse, *Peromyscus*, he did so with the object of comparing them with the behaviour of variations in the geographical races, and therefore only considered those correlations which were independent of general size of the body. In the present material this would disqualify all correlations which were reduced to insignificance in the partials. Sumner obtained a well-marked relationship between lengths of tail and foot, which might be comparable with a pair of characters like leaf breadth and scape thickness. He also found a moderate correlation (of about +0.3) between shade of pelage and width of tail stripe, but his other chosen characters gave no correlation.

The twenty plantain characters have so far been surveyed irrespective of their application to racial differentiation. Only two correlations have been found which seem to be entirely independent of collateral growth. The first connects time of flowering with habit (§ 1), for earliness is shown to be associated with decumbency, or lateness with erectness, in a given population. It should not be assumed, however, that this tendency will necessarily appear as a rule among habitat populations. The effects have not yet been examined critically, but it may be stated that the times of flowering are distinctive characteristics of populations, and that the erect plants of one population may flower earlier than decumbent plants of another. The second non-developmental relationship occurs between plant habit and scape length (§ 3), whereby erect types tend to have longer, or decumbent types shorter, scapes; and it has already been pointed out that there may be an adaptive value in this tendency. But length of scape is a function of habitat conditions (Gregor, 1938), and varies considerably from habitat to habitat. The scapes of decumbent plants in one population may, indeed, be twice as long as those of erect plants in another.

It has been shown by Gregor (1938) that characters of size and habit of growth are sensitive to environmental selection in the sea plantains. He suggests that populations have attained their present composition largely because seed production has been favoured in those plants which grow to a length or habit which is optimal for the habitat conditions. Thus scape length, habit of growth and, no doubt, time of flowering, have adaptational value for particular environments. Trends of correlation between these characters are interesting, since they suggest linkage of some of the responsible factors. The correlations of time of flowering and habit with other characters present little of note, but since scape length is mainly a pronounced expression of plant growth, its relationships with characters on remote organs, some of which may be used to distinguish taxonomic units, are of great interest.

The classifications given by Fernald (1925) and Hegi (1906-) for the *Plantago* spp., *P. maritima*, *P. serpentina*, *P. carinata* and *P. alpina*, are partly based on colour and pubescence characters, partly on certain absolute measurements and ratios. These include the lengths of the anther and its subulate tip (for geographical races); the proportions of length : breadth of the sepal and the bract; the proportions of the lengths of sepal : bract; the length of the spike and its proportion to scape length. The present studies are not designed to test the validity of such taxonomic distinctions, nor to determine whether they are the expression of a few large variations or of the accumulation of many small ones. Though the samples of cross-fertilizing species are undoubtedly heterogeneous, they are unlikely to contain more than a small part of the total potential variability of the group. Some light may be thrown, however, upon the conditions under which the diagnostic characters must be exhibited and in particular on their reactions to growth.

For diagnostic purposes, an absolute measurement should be chosen either intentionally to gauge plant size, or to be independent of it. Among the present measurements, that of scape length is thought to be the best suited for determination of plant size. The anther measurements, on the other hand, are satisfactorily independent characters, for though variation naturally occurs, it is scarcely influenced by growth in other parts. Bract and sepal dimensions vary with flower size, but are liable, as shown in PMN 45, to be strongly affected by plant growth as well. Consequently as absolute measurements they are of doubtful value diagnostically in the plantain. Even more unreliable are measurements such as length of spike, where undoubted hereditary variations are greatly masked by the effects of plant growth, and only very large differences can be diagnostic for individual specimens. Multiplicity of observations, or comparative trial, naturally reduces the magnitude at which differences may be deemed significant.

A character composed of two measurements expressed as a ratio might be expected to be relatively unaffected by plant growth, but it has been seen that such freedom is not absolute. The diversity of results obtained with the indices of sepal and bract (§§ 11 and 14) suggests that more thorough examination should be made of these ratios and others such as bract length : sepal length and scape length : leaf length. Further studies have already shown that the coefficients cover wide ranges,

but that there seems to be some particular form of behaviour for each ratio. Thus the two suggestions raised by the present data are confirmed, for sepal index is more strongly correlated with breadth than with length in each of eleven samples, and bract index shows the opposite tendency by being more highly correlated with length than with breadth in all except PMN 45.

Confining the discussion to the present data, three conditions may be described. First, there is that of the bracts of PMN 45 (§ 14). Here the correlation between length and breadth is high, $+0.70$, and between length and index even higher, $+0.76$, but instead of the correlation between index and breadth being inverse as occurs in the other samples, it is positive though insignificant ($+0.15$). The index is characterized by showing a series of strong positive correlations with size characters. Length is strongly correlated with most of the characters, as also is breadth though to a lesser degree. The influence of breadth can be practically discounted because of its feeble relationship with index. It is interesting to note that length is considerably more variable than breadth (Table XXII); indeed its average increase for unit increase in breadth is greater than the mean index value, $2.04 : 1$. This is shown by the regressions of length and breadth:

$$L = -0.25 + 2.25B; \quad B = +0.72 + 0.22L.$$

Consequently there is no difficulty in seeing how a small positive correlation between index and breadth can accompany a high correlation between length and index.

For the second example the bracts of PMN 44 may be taken. Here the correlation between length and breadth is moderately strong, $+0.54$, and between length and index similar, $+0.59$, but between index and breadth there is a distinct inverse correlation of -0.31 . Length and breadth show positive correlations with all the size characters, sometimes the one and sometimes the other having the stronger relationship. Except with sepal length and sepal index, the bract index shows no significant correlations. The effects of length and breadth thus tend to cancel out. Nevertheless there is a tendency for index to be more affected by length, which gives its correlations a positive bias. The sign of the index correlation is reversed only when the corresponding breadth correlation is strong. The variability of length is again greater than that of breadth, but the difference is not so pronounced as in PMN 45. The regressions of length and breadth are

$$L = +0.93 + 1.45B; \quad B = +0.63 + 0.20L.$$

Thus the increase of length by 1.45 for unit increase in breadth does not exceed the mean index value of $2.27 : 1$. It will be seen, therefore, that positive correlation between length and index can exist in conjunction with negative correlation between index and breadth, at least when the length-breadth correlation is not too strong.

Passing over intermediate conditions in the bracts of PMN 63 and sepals of PMN 44, the behaviour of the sepals in PMN 63 may be taken as a third example. Here the correlation between length and breadth is only $+0.30$, and between length and index hardly more, $+0.35$, but between index and breadth there is a strong inverse correlation of -0.77 . The correlations of breadth with size characters

Table XXII. Statistics of characters

Character	Means			Standard deviations			Coefficients of variation			No. of plants
	PMN 63	PMN 44	PMN 45	PMN 63	PMN 44	PMN 45	PMN 63	PMN 44	PMN 45	
FIG	6.61 ± 0.167	5.06 ± 0.154	2.88 ± 0.126	1.682	1.558	1.249	25.5	30.8	43.1	99
SpD	14.26 ± 0.187	13.65 ± 0.219	11.95 ± 0.191	1.884	2.193	1.543	13.2	16.1	12.8	65
HbG	2.85 ± 0.047	2.92 ± 0.073	1.97 ± 0.083	0.473	0.737	0.796	16.6	25.2	40.0	93
ScS : H	1.30 ± 0.019	1.26 ± 0.018	1.41 ± 0.033	0.188	0.182	0.320	14.4	14.4	22.7	93
LfS : H	3.18 ± 0.069	3.80 ± 0.097	3.93 ± 0.119	0.700	0.981	1.151	22.0	25.8	29.5	94
ScV	7.34 ± 0.290	5.70 ± 0.178	2.76 ± 0.189 l.	2.929	1.795	1.820	39.9	31.5	66.0	93
AL	2.19 ± 0.019	1.91 ± 0.017	2.36 ± 0.027 mm.	0.104	0.169	0.193	8.9	8.8	8.2	50
ATL	0.29 ± 0.006	0.28 ± 0.005	0.27 ± 0.008 mm.	0.056	0.052	0.060	19.5	18.6	22.2	50
SepL	2.76 ± 0.022	2.45 ± 0.024	2.62 ± 0.027 mm.	0.222	0.241	0.191	8.0	9.8	7.3	50
SepB	1.07 ± 0.013	1.06 ± 0.012	— mm.	0.130	0.118	—	12.2	11.1	—	—
SepLx	2.60 ± 0.032	2.35 ± 0.028	—	0.326	0.280	—	12.6	11.9	—	—
BrL	3.17 ± 0.037	2.60 ± 0.037	2.69 ± 0.050 mm.	0.370	0.369	0.403	11.7	14.2	15.0	65
BrB	1.12 ± 0.012	1.15 ± 0.014	1.32 ± 0.016 mm.	0.126	0.137	0.127	11.3	12.0	9.7	65
BrLx	2.85 ± 0.031	2.27 ± 0.028	2.04 ± 0.026	0.316	0.282	0.213	11.1	12.4	10.4	65
ScL	42.50 ± 0.600	41.15 ± 0.464	35.75 ± 0.755 cm.	6.055	4.635	7.278	14.3	11.3	20.4	93
SpL	9.85 ± 0.215	9.99 ± 0.202	11.44 ± 0.290 cm.	2.166	2.023	2.800	22.0	20.3	24.6	93
ScTh	1.44 ± 0.022	1.60 ± 0.024	1.81 ± 0.033 mm.	0.225	0.237	0.314	15.5	14.8	17.4	93
LfL	22.32 ± 0.457	19.18 ± 0.351	11.22 ± 0.463 cm.	4.620	3.544	4.508	20.7	18.5	40.3	95
LfB	5.98 ± 0.161	6.25 ± 0.147	4.52 ± 0.183 mm.	1.621	1.485	1.782	27.1	23.8	39.6	95
LfTh	0.83 ± 0.013	0.87 ± 0.016	0.97 ± 0.017 mm.	0.135	0.161	0.171	16.3	18.5	17.5	95

generally exceed those of the length, and the corresponding index correlations duly show changed signs. The influence of breadth is also enhanced by a distinct bias. Breadth now has the greater variability (Table XXII), that of length being low. The regressions of length and breadth are as follows:

$$L = +2.21 + 0.514B; \quad B = +0.58 + 0.176L.$$

There is thus only half a unit increase in length on the average for unit increase in breadth. Such correlation as exists between length and index does so in spite of the low regression and on account of the comparative independence of length and breadth.

The examination of these data has clearly indicated that the question of growth affecting the ratio requires further investigation. Certain inferences may meanwhile be drawn from the limited illustrations provided by these samples: (1) A ratio may or may not be greatly affected by size of other parts of the plant. (2) A ratio may be correlated in various ways with one or both of the characters forming its terms. (3) Correlation between one or both terms and some other size character is a factor determining the correlation of the ratio with that character. (4) The latter relationship may also be affected by disparity between the strengths of the correlations of the two terms with their ratio; a "bias" occurring in favour of the more strongly correlated term. (5) A condition under which the ratio would be independent of size might be established if (a) the variability of the two terms were similar; (b) each term were correlated with the ratio to a similar extent, the second term inversely; and (c) each term were to form correlations of equal magnitude with other size characters. (6) Deviations from the theoretical conditions of (5) lead to indirect correlations being established between the ratio and other size characters. These may be changed in sign under the influence of the second term.

To amplify these records it may be mentioned that cases have been obtained where the ratio was negatively correlated with both its terms (e.g. in scape length : leaf length). In conclusion it may be inferred that the ratios, despite the limitations discussed above, seem to be less affected by growth than the characters comprising their terms, so that there is at least some advantage in using them for taxonomic description.

SUMMARY

Samples of 102 plants each of two races of *Plantago maritima* L. and one of *P. serpentina* All. were grown in an experimental garden, and twenty characters were examined for all possible direct correlations. Various intensities of correlation between measurements showed the effects of size on different organs. Bracts and sepals form a partly independent region of growth, but, in one sample, plant size affected all the measured parts except the anthers. A common trend of growth in thickness was traced in the relations of scape thickness, leaf breadth and sepal breadth, but was indistinct in bract breadth and absent from leaf thickness. Two correlations appeared to be due to causes other than growth. The habit of the scapes was associated (1) with time of flowering, for in a given population decumbent

types tended to be earlier and erect types later; and (2) with scape length where erect types tended to be longer than decumbent. Adaptational value can be assigned to the latter relationship.

For diagnostic purposes, absolute measurements should be either indicative or independent of plant size. In sea plantains, scape length and the anther measurements fulfil these conditions respectively. When a character is formed by expressing two measurements as a ratio, it might be expected to be relatively independent of the growth of other parts. Under some circumstances this is the case, but under others there may be considerable growth effects. The measurements forming its terms can show various states of correlation with the ratio and with one another. The length : breadth ratios under examination typically showed positive correlation with length and negative with breadth, the second term. The correlations which these terms formed with size characters were impressed upon the relationships of the ratio in opposite ways, so that if equal in strength they cancelled out. But when one exceeded the other the ratio showed a correlation roughly corresponding with the excess, the sign being altered if the influence came from the second term. In addition, the measurement more strongly correlated with the ratio, apparently on account of higher variability, seemed to exert a greater effect than the other. A bias in its favour was given to correlations between the ratio and other size characters. In one sample both terms were positively correlated with the ratio which was greatly influenced by size. Instances are also known where both correlations are negative, so that it seems unsafe to assume that a ratio will be unaffected by growth without some knowledge of these interactions.

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THE MOVEMENTS OF THE AIR PORES OF *PREISSIA QUADRATA* (SCOP.)

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(With 2 figures in the text)

THE Marchantiaceae are peculiar among liverworts in possessing an assimilatory tissue with air spaces, in many ways comparable with that of a green leaf. This tissue is divided by septa into air chambers, each of which communicates with the outside by a small air pore through the upper epidermis. In most genera the air pores of the thallus are of simple structure, but in the archegoniophores of many genera and in the thallus of a few, the air pores are more complex. These complex pores are barrel-shaped, and in some at least of these genera the pore is capable of being closed in a manner which recalls the stomata of a leaf.

The present investigation shows that although, as previous workers have shown, the air-pore movements are comparable in some respects with the movements of stomata, there is an interesting and fundamental difference in the mechanism of the movement.

TECHNIQUE

The *Preissia quadrata* material used was cultivated in a frame in Reading from plants obtained from rocks on Cader Idris, North Wales, in July 1936. Material collected at the same time and preserved in spirit was also used.

The air-pore movements are observable in intact pieces of the thallus, but can be better observed in pieces of the epidermis sliced off with a razor, mounted upside down and allowed to dry. They can be observed much more closely and conveniently if the epidermis is mounted in liquid and the air from the pores is removed by evacuation. Various mounting media were employed.

As will be explained, the life of the material has no effect on the result, and for certain experiments spirit or boiled material was used.

Vertical sections were cut from preserved material with a hand razor. They need to be thick enough to contain the essential cells in an uninjured state, but thin enough to see clearly. These sections were irrigated in the same way as the slices of epidermis.

STRUCTURE

The air pores of *Preissia* and of other liverworts have been described by Kammerling, Goebel and others. In *Preissia* the pore is formed by a barrel-shaped tube of cells of four or five tiers (Fig. 1). The upper aperture is always wide, but the inner is narrower and capable of being closed. It is narrower because each cell of the basal tier, which we call "motor cells", has a papilla which projects freely inwards and

constricts the aperture so that the inner aperture often appears as a star-shaped slit. The wall of this papilla is thinner than that of the rest of the motor cell. There are from three to six of these motor cells, but four is the commonest number. The size of the inner aperture varies between fairly wide limits in different pores in the same piece of epidermis, even when mounted in water. The changes in size of the aperture are caused by movements of the papillae of the motor cells. The epidermis in our material was scarcely cutinized and had chlorophyll, but the air pore cells, though they contained protoplasm and a vacuole, had no chlorophyll.

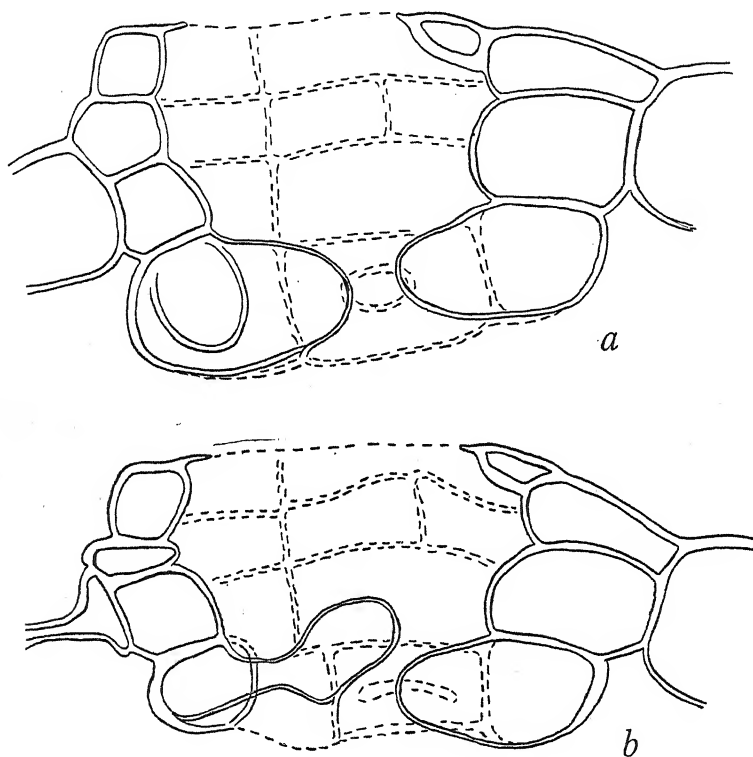


Fig. 1. Vertical section of the air pore of *Preissia* showing the tier of motor cells: (a) mounted in water; (b) mounted in strong glycerine. The motor cell on the right is not functioning, presumably because of injury. $\times 800$.

MOVEMENTS AND MECHANISM

Fig. 2 shows a typical pore in the open and closed conditions. It is evident that the movement is due to changes in the shape of the papillae, and not to a general constriction of the lowest tier. In surface view, irrigation with strong glycerine causes the papillae to increase in surface area. In the specimen figures, this has reduced the area of the pore from 4.69×10^{-4} to 1.25×10^{-4} sq. mm. In other pores the aperture remains wider than in the figured specimen, while in yet others the papillae overlap and close the aperture completely. From the vertical sections it is seen that

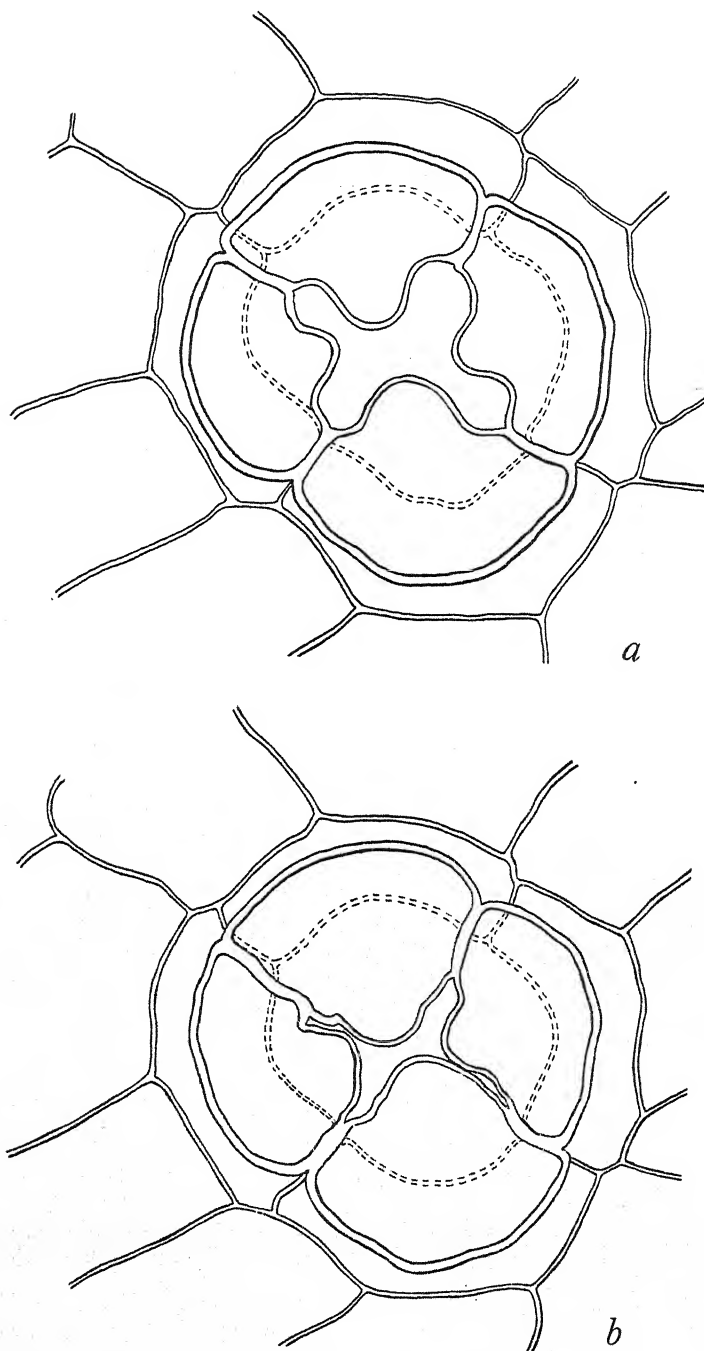


Fig. 2. A typical air pore of *Preissia* viewed from below, showing variations in the size of the aperture due to changes in shape of the papillae of the motor cells: (a) in water; (b) in strong glycerine. $\times 800$.

the motor cells flatten in glycerine, the extension of the papillae being caused by a decrease in volume of the motor cells.

These movements are most naturally observed in fresh material, but can also be readily observed in old spirit material and in boiled fresh or spirit material. Irrigation with strong alcohol has been observed to cause closing in some cases. This will be mentioned later.

It must be pointed out that strong glycerine is needed to cause maximum closure; 50 % glycerine, molar cane sugar, or dilute potassium nitrate solution which other authors employed only cause partial closure. Another remarkable feature is that even in strong glycerine the pore soon begins to recover and open. It takes about ten seconds for the pore to close when irrigated with strong glycerine, and appreciable reopening takes place within another ten seconds.

In living material, glycerine causes plasmolysis of the epidermal cells. The motor cells do not plasmolyze, they flatten, and after reopening of the pore has taken place, the protoplasm of the motor cells remains in a permanently shrunken condition.

DISCUSSION OF MECHANISM

The pore of *Preissia*, unlike an ordinary stoma, is open in the "position of rest", i.e. the condition of the dead cells soaked in water. Under these conditions the pressure of liquid inside the cell is presumably the same as outside, and the stresses in the walls are at a minimum. Glycerine, which withdraws water from the cell, causes the wall to flatten, but it does not cause plasmolysis: in this again the *Preissia* pore differs from a stoma. Evidently this flattening and the subsequent recovery indicate that the cell wall must be readily permeable to water but less permeable to glycerine.

The behaviour of the pores on addition of strong alcohol is anomalous. Sometimes the addition of alcohol to pores mounted in water causes them to open, and sometimes pores similarly mounted in water will close if strong alcohol is added. This seems to indicate that some factor other than permeability is involved.

According to rough experiments with strong sugar solution and potassium nitrate solution, these are similar to glycerine in their effect on the air pores.

Under natural conditions, closure of the air pore is brought about by a gradual drying of the tissues, which leads to loss of water from the motor cells, resulting in flattening of the papillae and closing of the pore. Under these conditions recovery occurs when the epidermis is again supplied with water.

The deformation of the motor cells has a close resemblance to the changes of shape described by Haberlandt in the guard cells of the moss *Mnium cuspidatum*, though the mechanism is quite different.

EFFECT OF MOVEMENTS

Although the closure of the aperture observable under the microscope is striking, the pore is somewhat complicated, and it is not safe to assume without experiment that the air passage has really been constricted or closed. The following

experiment indicates that the observed closure of the pores causes reduction in the air channels.

The under surface of the thallus was sliced away with a razor until the air chambers were pierced from below, and this preparation of the upper epidermis with the air pores intact was cemented on to a small porometer tube with vaseline. This connexion can be made quite airtight by melting the vaseline with a warm needle. It is necessary to prepare the thallus in this way because there is no lateral communication between neighbouring air chambers. A porometer set up in this way will allow air to pass through at a rate suitable for measurement. To examine the behaviour in a damp atmosphere the thallus is surrounded by a piece of glass tube plugged with wet cotton-wool.

In damp air a fairly steady rate of flow of air was obtained. On allowing the thallus to dry, the rate of flow at once begins to fall off and will ultimately become very low indeed, as the preparation dries up. In this state it will not recover, but if it was only allowed to dry for a short time and then the moist atmosphere replaced, recovery occurs and the flow returns to a steady rate.

In one experiment the rate was constant in damp air over nearly two hours: on thirty minutes' drying it fell to nearly half, and on restoration to damp air it returned to a new steady rate, only two-thirds of the previous rate. Then on drying again the rate fell to a fifth in the course of 90 min. Even after a night's exposure to damp air it did not recover. It seems peculiar that this new constant rate was not found to be the same as the previous one, but may be quicker, though apparently it is more often slower.

It should be pointed out that a large proportion of experiments were rejected. In these experiments there was either no flow or an unreasonably rapid flow. We consider that these unsatisfactory results were due to blocking with vaseline or else tearing of the epidermis. It must be realized that the pieces used are necessarily minute, the region where the air chambers are cut open being about 1 sq. mm. in area, and that the air chamber zone is only about $\frac{1}{10}$ mm. thick.

EFFECT OF THE AIR PORES ON THE RATE OF TRANSPIRATION

As a preliminary experiment, the transpiration was examined as follows. A piece of thallus, vaselined by its underside and margins to a cover-slip, was weighed at periods of 5 min. upwards, and so the loss of weight by transpiration was determined. During the experiment it was kept at approximately constant temperature and humidity in a balance chamber which contained a desiccator. The loss of weight was found to follow a fairly steady curve which flattened off as the thallus became completely dried.

To determine the effect of the air pores on this rate of water loss, the following experiment was carried out. Two pieces of thallus of approximately the same size, from the two forks of a branched thallus, were taken and each was mounted on a coverslip as described above. Each was counterpoised on a balance, and the loss of weight was followed. For a little over an hour, the two pieces lost weight at nearly

equal rates. Then the two coverslips were both removed to the laboratory atmosphere, and the air pores of one piece were quickly stabbed with a sharp needle under a binocular microscope. The other piece was left uninjured. Both pieces were then returned to the balance chambers. The rate of loss from the stabbed piece immediately increased. Before the stabbing, both pieces were losing weight at 8.3 mg./hr. After stabbing, the rate of loss of weight from the stabbed specimen rose in the first hour to 16.3 mg., while that of the unstabbed specimen had fallen slightly to 8.0 mg./hr. This increased rate of loss from the injured specimen resulted in its reaching the air-dry condition more rapidly than did the uninjured specimen, though the total loss of weight from each piece was about the same.

The epidermis as a whole with uninjured air pores would therefore seem to act as a certain check on transpiration.

DISCUSSION OF PREVIOUS WORK

Goebel (1882) appears to have been the first to describe the movements of a Marchantiaceous air pore. He observed it in *Preissia* and supposed that the mechanism resembled that of a stoma.

Kamerling (1897) discovered the movements independently and described them in some detail, but assumed that the movement was caused by a turgor change and thus was dependent on life. He thus confined himself to the material of which he had live specimens, and stated, for instance, that he could make no investigation of *Preissia* because he had only spirit material. He chiefly used *Fimbriaria stahliana* which has barrel-shaped air pores on the head of its archegoniophore. In this plant, the pore is formed by about six tiers of cells, the inner two or three of which are immersed. The end cells are the motor cells. They are somewhat rounder than those of *Preissia* having no papillae in the condition of rest. He immersed specimens in 6 % potassium nitrate solution, and caused partial closure, and gives figures illustrating the change both in surface view and in section. He also states that alcohol causes closure. This is mentioned below.

We have not had access to *Fimbriaria stahliana*, but have examined another species of *Fimbriaria*, probably *F. borreyana*, cultivated in Reading, which produces air pores closely resembling Kamerling's figures. Here also, partial closure is caused by weak plasmolyzing solutions, while concentrated glycerine causes complete closure. This is brought about by a shrinkage in the motor cells which become wedge-shaped, the inner wall bordering the air pore being thus caused to bulge and obliterate the pore. The movement is thus fundamentally similar to that of *Preissia*. Here also the movement is independent of life, occurring very well in old spirit material.

Kamerling observed that the application of alcohol causes the pores to shut. We found that when strong alcohol was added to material previously mounted in water, the pores do not behave uniformly. Some remain open, some close slowly, and some close rapidly and begin to open almost immediately. We presume that Kamerling was dealing with the type of pore which closes, but that he did not watch it long enough to observe the reopening.

BIOLOGY

Goebel, Kamerling, and Cavers all suppose that the closure of the air pores has a biological advantage in limiting the transpiration in a plant in a situation which sometimes gets dry. They give no experimental evidence in support of their views, but the experiments given here do suggest that the shutting of the pores is likely to cause some diminution in the rate of transpiration under natural conditions.

It is very difficult to find evidence that this power of closing is of value to the plant in nature. Cavers and Goebel, indeed, speak of the plant as occupying places which sometimes become dry. Though we are hardly in a position to discuss the relation of these air-pore movements to the ecology of *Preissia*, we consider that this is in any case not obvious. In Britain, *Preissia* appears to be common chiefly on calcareous rocks in the west, especially on mountains. It is also found occasionally in damp dune slacks. It is rare or absent in the drier eastern and midland counties, and it may thus be said that its distribution coincides with the region of higher humidity. Other Marchantiales, however, are not so confined. *Lunularia*, *Marchantia*, and *Conocephalus*, which have immovable air pores, grow in all parts of the country in a variety of situations. In culture, too, though *Preissia* shows some tolerance of drought, it is certainly no more tolerant than the above three genera, or *Reboulia*, which again has simple air pores: indeed, it appears to be hardly as resistant.

We wish to acknowledge our indebtedness to Prof. T. M. Harris for suggesting this problem, and for his continued help and criticism throughout the preparation of this paper.

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THE STRUCTURE AND DEVELOPMENT OF THE HYDATHODES OF *SPARTINA* *TOWNSENDII* GROVES

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(With 19 figures in the text)

INTRODUCTORY

THE sea cord-grass, *Spartina Townsendii* Groves, grows abundantly in muddy, coastal and estuarine soils which are regularly inundated by the tides, particularly on the south coast of England. The body of the plant consists of a horizontal fleshy rhizome invested by scale leaves and ending in a large bud, which in the spring grows out of the mud to become the annual aerial shoot of the plant. The axis of the latter is vertical, terminates in the inflorescence, and is completely enclosed in its lower half by the sheaths of the foliage leaves, which are borne alternately in two opposite rows. The mature leaf consists of a split sheath which very closely clasps the axis and the sheath of the next leaf above, a lamina attached to the top of the sheath and carried at a wide angle to the vertical, and thirdly a soft hairy ligule closely pressed to the axis at the junction of the sheath and lamina. The lamina and sheath are connected by a cartilaginous pulvinar articulation.

The general anatomy of *Spartina Townsendii* has been described in detail by Sutherland & Eastwood (1916).

LEAF STRUCTURE AND LOCATION OF HYDATHODES

Hydathodes are absent from the rhizome, from that part of the stem invested by leaves, and from the inner surfaces of the leaf sheaths, but they are found with varying frequency in all other aerial parts of the grass with the exception of the flower itself. A few are usually present on the barren glumes, but we have yet to find hydathodes on the flowering glume or palea. The observations described here have been made from hydathodes occurring on the lamina of the leaf, since this material is the most convenient with which to work.

The adaxial and abaxial surfaces of the leaf differ strikingly. The abaxial surface is smooth. On the adaxial surface, however, there are some forty flat-topped ridges, each about 200μ in width at the base but tapering slightly upward and more than half the total thickness of the leaf in height. These run parallel along the full length of the lamina. In the mesophyll at the base of each ridge is a vascular bundle

enclosed by two eccentric sheaths of tissue, the inner one fibrous and the outer one of large cells with colourless contents. This outer sheath is extended on the adaxial side to form the core of the ridge immediately above. The assimilating cells with which the hydathodes are always associated occur immediately below the epidermis in zones which run the full length of the leaf. As seen in transverse section each zone extends from a point just below the flat top of a ridge, down the flank, beneath the groove and up the flank of the adjoining ridge to a similar point near the top. They extend inward as far as the core of colourless cells, but beneath the groove to within a single cell of the lower epidermis (Fig. 1). The epidermal cells of the leaf are of two kinds, both roughly rectangular in shape and arranged in unbroken rows from one end of the lamina to the other. The commoner type of cell is greatly elongated in the direction of the long axis of the leaf and the average dimensions are 130μ in length, 30μ wide and 30μ in depth. The other type of cell is of the same width and depth but only about 10μ in length; such cells usually occur singly between the ends of the long epidermal cells but are sometimes found in pairs (Figs. 6, 8). The lateral walls which come into contact with other epidermal cells are sinuous, more conspicuously so on the adaxial than on the abaxial side of the leaf. The cuticle on the adaxial epidermis is raised into large numbers of small papillae, the average number being about fifty to each long epidermal cell, but the abaxial cuticle is quite smooth.

The hydathodes are epidermal structures not connected directly with the water-conducting system of the plant. They are always in direct contact with the assimilating tissue on the adaxial side of the leaf and on the abaxial side are only separated from it by a single layer of large cells with colourless contents. On the adaxial side they are to be found in a single longitudinal row on each side of the ridges, about six epidermal cells from the top and always higher than the stomata. Hydathodes in the same row are separated by between two and five long epidermal cells, i.e. by $200-500\mu$. On the abaxial side they occur in rows lying between the vascular bundles, sometimes a single row between each pair of bundles, sometimes two rows. The hydathodes on this surface are more widely spaced than on the adaxial surface. The relative frequency is indicated by the figures obtained when the number of hydathodes in equal areas of epidermis on the two sides of the leaf were counted. There were forty-two on the adaxial surface to twenty-seven on the abaxial surface.

THE STRUCTURE OF THE MATURE HYDATHODE

The complete hydathode involves four epidermal cells so arranged as to leave a roughly cylindrical opening in the epidermis, here termed the *well* of the hydathode, and a specialized structure of two cells constituting the hydathode proper. The latter will be described in detail first.

The larger basal cell of the hydathode is attached to the four surrounding epidermal cells but is sunk in the tissue of the leaf so as to leave a roughly cylindrical depression in the epidermis above it. This depression is delimited by the end walls of the two epidermal cells in the same longitudinal row and by part of the lateral walls of epidermal cells in the adjoining rows (Figs. 2-4). The upper and smaller of

the two specialized cells is the *cap cell*; this may be regarded as an outgrowth from the outer wall of the basal cell, since, apart from its attachment to the basal cell, it is quite free in the well of the hydathode which it more or less fills (Figs. 2, 4).

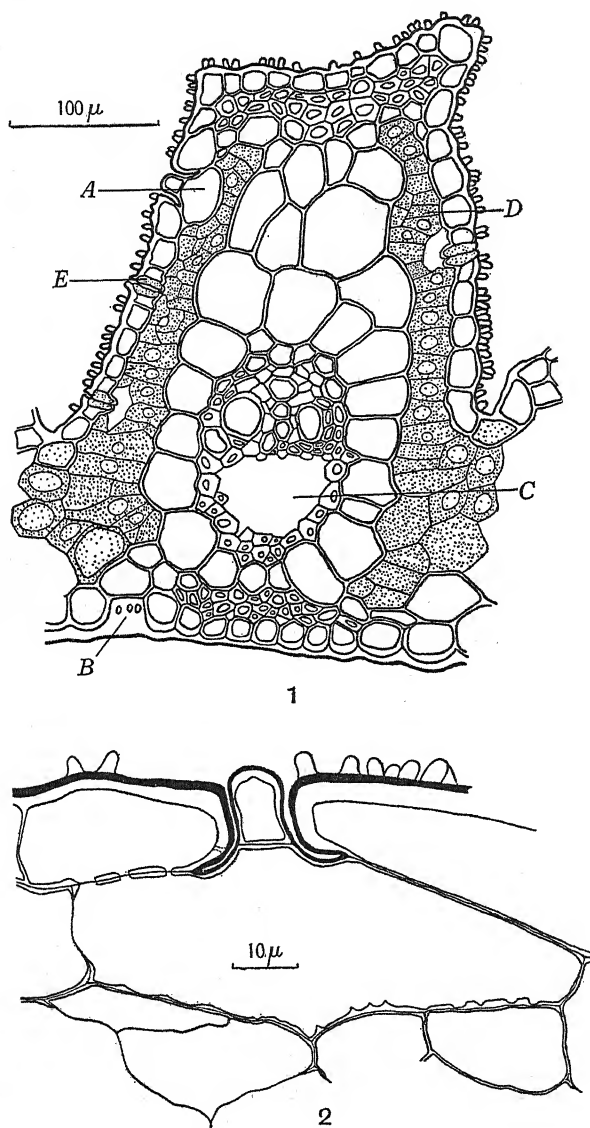


Fig. 1. Transverse section of part of the mature leaf to show the general distribution of tissues and the location of the hydathodes. *A*, adaxial hydathode; *B*, well of abaxial hydathode; *C*, phloem—detail omitted; *D*, mesophyll; *E*, stoma.

Fig. 2. Longitudinal section through hydathode of adaxial epidermis to show sections of pits leading from the basal cell into the epidermal and mesophyll cells.

The basal cell is spindle-shaped with its long axis directed along the length of the leaf. Its dimensions are approximately 100 μ in length, 30 μ in width and 30 μ in

depth. Because it is immersed in the tissue of the leaf its upper half abuts on parts of the inner walls of the four epidermal cells which define the well of the hydathode. The lower half of the basal cell comes into contact with many assimilating cells of the mesophyll in the case of hydathodes on the adaxial surface of the leaf and with large cells of the water-storage type which sheath the vascular bundle in the case of those on the abaxial surface. Communication with both epidermal and mesophyll cells is made by large variously shaped pits (Figs. 5, 7, 9, 14). The large mesophyll cells which surround the basal cell on the abaxial side of the leaf have walls as thick as those of the basal cell itself and the pits can be seen in both (Fig. 14). The assimilating cells which surround the basal cell on the adaxial side have extremely thin walls and the pitting is much less evident (Figs. 2, 7). The wall of the basal cell is composed of cellulose with the exception of an annular segment of the outer portion between the line of its attachment to the four epidermal cells and its attachment to the cap cell, which is also cuticularized. This cuticularized area is exposed to the cavity of the well (Figs. 2, 3, 5, 10, 14).

The cap cell is dome-shaped with the flat side applied to the outer wall of the basal cell. Its height is about 10μ and the diameter of its base about $12-13\mu$. The cellulose wall is strongly cuticularized on the exposed outer surface and is devoid of pits. That part of the wall which separates the cap cell from the basal cell is also quite devoid of pits (Figs. 2, 14). Both the cap cell and the basal cell are typically glandular in that each is full of protoplasm and has a large strongly staining nucleus (Fig. 3).

The epidermal cells framing the well of the hydathode have external walls which are very thick and lateral walls which are very thick at the exterior but become progressively thinner toward the interior. Thus the walls of the actual hydathode well are thick at the mouth of the opening and become thinner toward the line of articulation with the basal cell where they are sufficiently thin for the joint to be somewhat flexible. The outer surface of the well has a thick cuticular covering which, apart from certain perforations described below, forms a complete coating over the well

Legends of Figs. 3 to 9

Fig. 3. Transverse section of mature hydathode. *A*, cap cell; *B*, basal cell.

Fig. 4. Surface view of abaxial epidermis showing the well and pits in the wall of the basal cell. *A*, wall of hydathode well; *B*, wall of cap cell; *C*, contents of cap cell (shaded); *D*, pits connecting basal and epidermal cells.

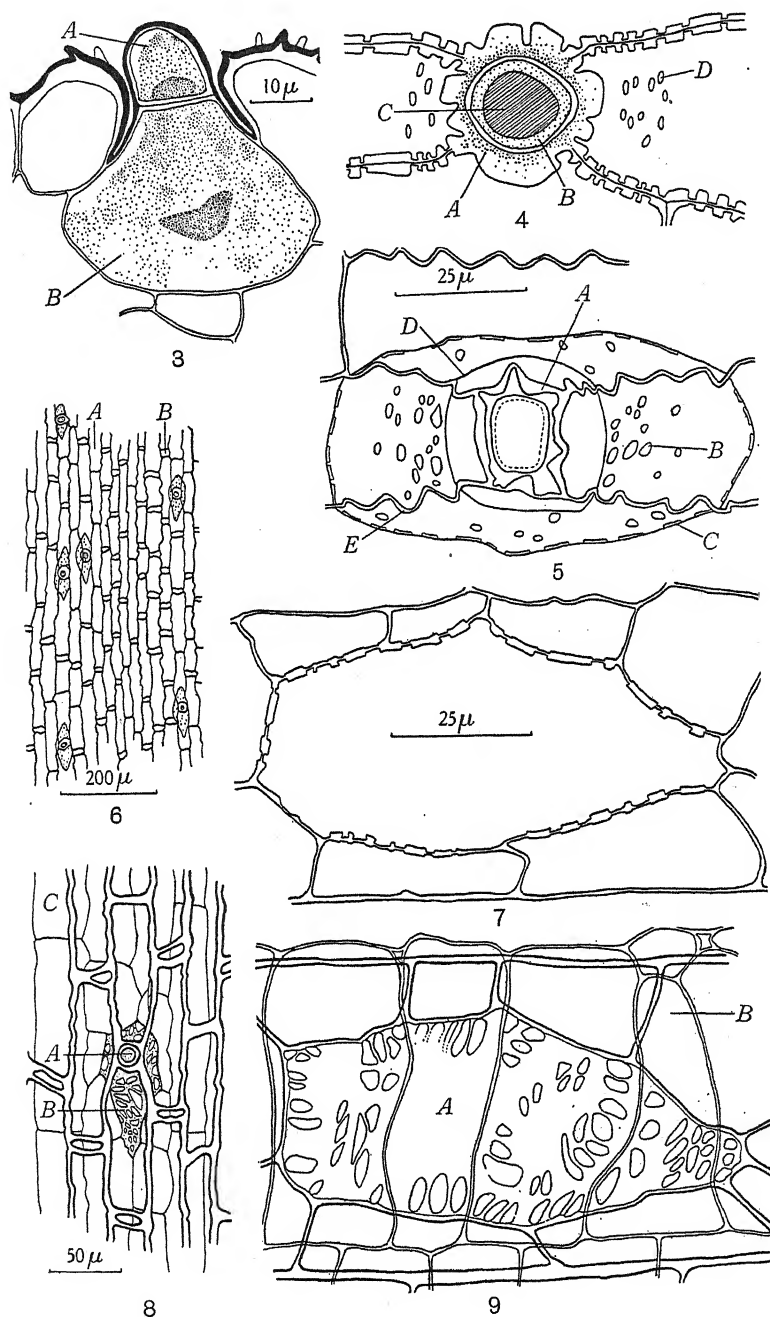
Fig. 5. Composite diagram from three optical sections of the adaxial hydathode viewed from above. *A*, wall of well; *B*, surface view of pit connecting epidermal and basal cell; *C*, wall of basal cell in section, with pits communicating with mesophyll cells; *D*, line of articulation of basal cell with epidermal cells; *E*, lateral wall of epidermal cells.

Fig. 6. Surface view of the abaxial epidermis to show the location of the hydathode. *A*, long epidermal cell; *B*, short epidermal cell.

Fig. 7. Longitudinal section of the basal cell cut parallel to the leaf surface showing the pits in section.

Fig. 8. Hydathode in surface view seen through the abaxial epidermis. *A*, cap cell in well; *B*, basal cell of the hydathode showing pits communicating with the epidermal cells; *C*, mesophyll cell.

Fig. 9. Hydathode basal cell viewed through the underlying mesophyll cells, showing surface view of pits connecting them. *A*, basal cell; *B*, mesophyll cells.



Figs. 3-9

walls, the cap cell and the exposed annular part of the basal cell. Like the cellulose part of the walls the cuticle becomes progressively thinner from the mouth of the well inwards and is quite thin at the line of articulation (Figs. 2, 3, 10, 14). Treatment of transverse sections of the epidermis with Schultze's solution shows a thin layer of cutinized material immediately within the primary layer of the outer wall. This cutinized layer is somewhat thicker at the corners of the cells and extends for some distance inward along the lateral primary wall separating adjoining epidermal cells (Figs. 10, 14). It does not seem to be present in the walls which form the well of the hydathode, nor in the wall of the cap cell or that of the basal cell. The cuticle of the adaxial epidermis is thinner than that of the abaxial epidermis and unlike it is provided with papillae which closely border the opening of the well.

The walls of the epidermal cells are deeply pitted. In the thick outer walls there are numerous pits which are relatively narrow and penetrate no further than the primary wall separating the cellulose layers from the cuticle (Figs. 10-12, 14). In the walls bounding the well of the hydathode the pits are wider and pass through both the cellulose wall and the cuticle and are closed only by a very thin membrane in the region of the primary wall (Figs. 10-14). It has not been possible to determine the chemical nature of the pit-closing membrane with certainty. It seems likely, however, that this membrane is of a pectic nature like the primary wall, since, when seen in surface view in sections stained with Ruthenium Red, the pits have a pale pink colour. The surface of the cuticle within the wells of hydathodes in the abaxial epidermis of the leaf, forms a few, fairly extensive but very shallow depressions into each of which two or three of the larger pits open (Figs. 11-13). These depressions have not been seen in the adaxial hydathode where the well is much shallower and the cuticle thinner. The part of the pit which passes through the cuticle often becomes wider toward the exterior giving a trumpet-shaped appearance to the pit as a whole (Figs. 12, 13). In all there are twenty to thirty pits of various diameters opening into the well of the hydathode. The inner ones are oval and large, greater than 2μ in diameter, whereas the outer ones tend to be small and circular in section, e.g. $\frac{1}{2}\mu$ in diameter. The length of the pits naturally depends upon the thickness of the wall and so it is greater in the case of those opening into the outer part of the well. A pit opening into the outer part of the well of an abaxial hydathode measured 7μ in length, whilst one of the innermost in the same well was only 3μ long.

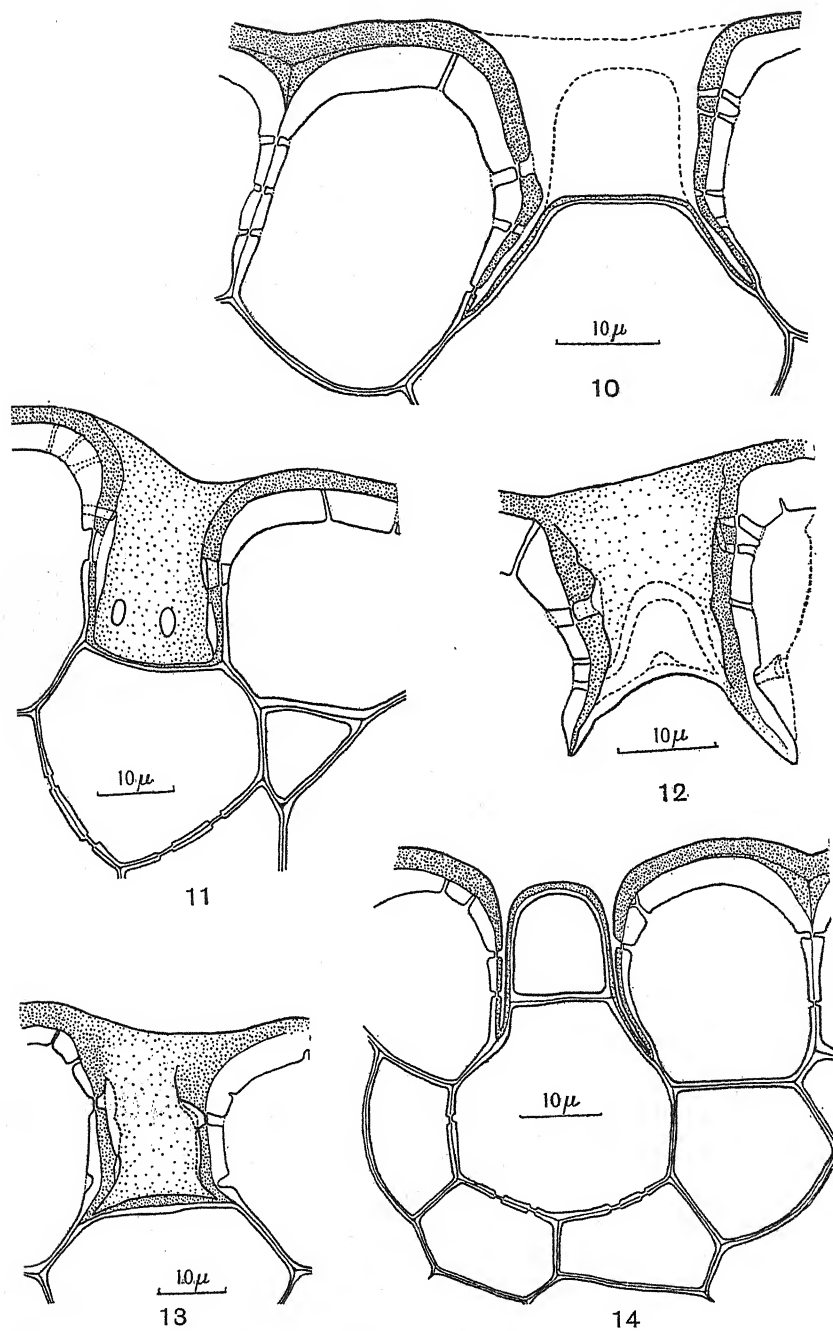
As the cap cell and the cuticularized area of the basal cell are both devoid of pits, it is presumed that the secreted salt solution passes from the epidermal cells into the well cavity of the pore through the pits in its sides.

Legends of Figs. 10 to 14

Fig. 10. The well of the hydathode and part of the basal cell in transverse section, showing pits opening into the well (diagrammatic). The cap cell is shown by a broken line since it was below the plane of the optical section in which the pits were visible.

Figs. 11, 12, 13. Various views of the well of the hydathode showing pits and pit openings in various positions (cuticle shaded).

Fig. 14. Transverse section of mature abaxial hydathode showing the pits opening into the well and pits in the wall of the basal cell and mesophyll cells beneath it (diagrammatic, cuticle shaded).



Figs. 10-14

THE DEVELOPMENT OF THE HYDATHODE

The two-celled glandular part of the hydathode is derived from a single epidermal initial cell which becomes distinguishable from the other epidermal cells whilst the young leaf is still in the bud. The hydathode initial grows more rapidly than the other epidermal cells and assumes a characteristic shape by the extension of the basal part into the mesophyll and the projection of the outer part beyond the surface of the epidermis. The nucleus is unusually large (Fig. 15).

The nucleus of the initial cell undergoes normal mitotic division (Figs. 16-18), one daughter nucleus remaining in the basal region of the young hydathode and the other passing to the outermost part of the cell. In cell division, which follows immediately, a cell wall formed in the plane of the leaf surface cuts off the knob-like projection of the initial cell to form the cap cell (Figs. 18, 19). The basal cell grows rapidly and soon reaches its final shape and size but the cap cell grows very little after cell division. Shortly after cell division, the well of the hydathode appears as a result of the overgrowth of the epidermal cells and the consequent sinking of the glandular cells into the tissue of the leaf until the cap cell no longer projects from the surface (Figs. 3, 19).

Cutinization of the leaf begins early. Before the majority of the hydathode initial cells have undergone cell division, small discontinuous areas of cuticle can be seen on the surface of the cap cell and the epidermal cells. The early stages in the development of the cuticle were best seen in sections which had been stained in a cold solution of Sudan III in a mixture of equal parts of 25 % glycerine and 25 % alcohol for 24 hr. and then mounted in glycerine jelly. The discontinuous areas of cuticle rapidly become larger until by the time the hydathode initials have divided there is a thin continuous cuticle covering the whole surface of the leaf and the cuticular papillae have appeared on the adaxial epidermis. The authors are not aware that the first appearance of cuticle in the form of discontinuous areas has been described previously. Development to the mature condition consists of the rapid deposition of cellulose on the inside walls of the epidermal cells, the cap cell and to a much less degree of the basal cell and also of a strong thickening of the cuticle particularly on the abaxial side of the leaf. The pits in the wall of the basal cell of the hydathode and those opening into the well become visible for the first time during this thickening process.

MODE OF ACTION OF THE HYDATHODE

The hydathode secretes salt solution of a fairly high concentration. Measurements made upon drops of solution from the leaves of pot-grown plants watered with a culture solution of approximately the same composition as sea water, using Barger's method gave a value of nearly $\frac{1}{2}$ *M* strength or about 25 g. per litre calculated as NaCl. The course which the liquid takes is not yet certain but the structure of the hydathode is suggestive of what happens. The fact that pits opening to the exterior occur only in the walls of the epidermal cells delimiting the well makes

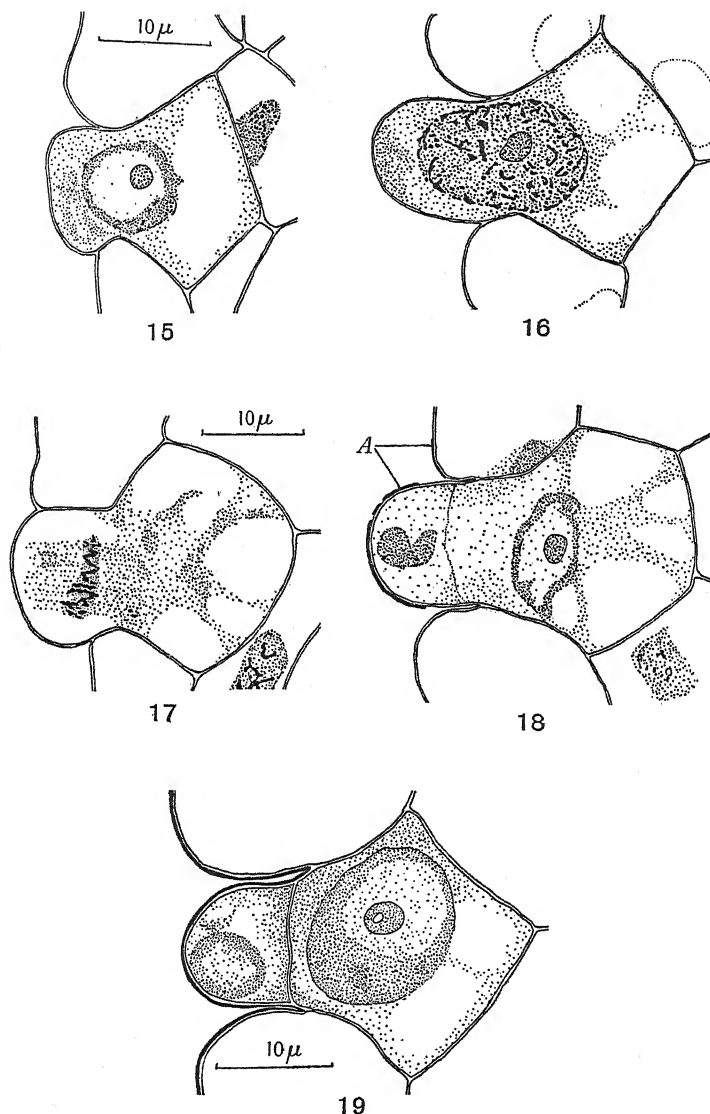


Fig. 15. Transverse section of young leaf and hydathode, showing nuclear resting stage in the initial cell.

Fig. 16. Transverse section of hydathode initial cell showing prophase of mitosis.

Fig. 17. Transverse section of hydathode initial cell showing metaphase of mitosis.

Fig. 18. Transverse section of initial cell showing cell division and the early stages in cuticularization. A, small discontinuous areas of cuticle.

Fig. 19. Transverse section of young hydathode in the two-celled stage, showing the wall separating the cap cell and the basal cell and a continuous though thin covering of cuticle.

it almost certain that the liquid emerges from these cells. If a leaf of a shoot standing in salt solution is enclosed in an air-tight chamber and observed under a microscope by means of an ultrapak lens, drops of liquid may be seen appearing very quickly on either side of the grooves of the adaxial surface. The glandular nature, size and freely pitted walls of the basal cell suggest that it acts as a centre into which the secreted fluid is drawn from the numerous mesophyll cells in contact with it. Presumably the salt solution passes from the basal cell to the epidermal cells and so out into the well of the hydathode.

The hydathode possibly acts as a valvular mechanism. The articulation of the basal cell to the four epidermal cells at the base of the well is thin enough to be flexible and so may act as a hinge. When the leaf is in a turgid condition we may suppose that the basal cell is distended and the well kept open so as to permit secretion from the pits. When the turgor of the leaf is reduced the rigidity of the epidermis would cause the well to close by bringing the walls of the epidermal cells into contact with the cap cell, which is the condition generally found in sections. We have not been able to confirm this mechanism by direct observation. The fact that secretion occurs freely when a detached leaf is placed with its cut end in water, indicates that the process is essentially an active one. Further work is necessary before the exact mode of action of the hydathode can be clearly understood.

SUMMARY

1. The structure, distribution and function of the hydathodes of *Spartina Townsendii* Groves are described.
2. The hydathode is an epidermal structure consisting of a well-like opening (the well) in the epidermis, bounded by four epidermal cells and two specially modified glandular cells (the basal cell and cap cell). The basal cell is much larger than the cap cell and is sunk in the assimilating tissue of the leaf with which and also with the adjacent epidermis it is connected by pits. The cap cell is attached to the outer surface of the basal cell and almost fills the well. Its wall is not pitted.
3. The walls of the epidermal cells which define the well of the hydathode are perforated by numerous pits which pass through both the cuticle and the cellulose part of the wall and are closed only by a thin membrane in the region of the primary wall.
4. The glandular part of the hydathode arises in the young leaf from a single epidermal cell which grows inward into the tissue of the leaf and then divides to form the basal and cap cells. Both cells have large nuclei and abundant cytoplasm.
5. The cuticle was observed to make its appearance in the young leaf in the form of small discontinuous areas which rapidly extended until a thin uniform covering was produced. The surface of the well and the cap cell are covered with cuticle in the mature hydathode.
6. The hydathode secretes a salt solution consisting mainly of sodium chloride with a concentration of about $\frac{1}{2}$ g.mol. per litre.

Structure and development of hydathodes of Spartina Townsendii 79

The work done by one of us (J. W.) was carried out whilst in receipt of a research grant from University College, Southampton. In conclusion the authors wish to express their gratitude for valuable suggestions to members of the staff of the Botany Department of University College, Southampton, and especially to Prof. S. Mangham whose advice and criticism has made this publication possible.

REFERENCE

- SUTHERLAND, GEO. H. & EASTWOOD, A. (1916). The Physiological Anatomy of *Spartina Townsendii*. *Ann. Bot., Lond.*, 30, 333-51.

REVIEWS

Plant Physiology. By N. A. MAXIMOV, edited by R. B. HARVEY and A. E. MURNEEK. 9 × 6 in. Pp. 473 + xviii with portrait frontispiece and 144 figures in the text. New York and London: McGraw Hill. 1938. 25s.

It was in 1930 that Prof. Maximov's text-book was first presented to Western readers at a time when such a book was greatly wanted. It has had a career of widespread usefulness and is now produced in a new form in harmony with certain developments in the author's views. The changes are considered great enough to require a change of title.

Prof. Maximov is anxious to present the plant as an integrated organism and to avoid a disjunctive treatment as far as possible. He now considers that the original work erred in this respect. Put into practice, the new attitude has resulted in two initial chapters on physico-chemical organization and general metabolism respectively. Respiration and growth follow, and then the "separate functions" precisely as before. In the final chapters synthesis is again the keyword in the treatment of development, correlation and the plant's relations to its environment. These last chapters are much enlarged from the corresponding sections of the earlier work, and more than anything else make the distinctive feature of the new one. The order of the chapters follows the annual cycle, but the advantage gained by introducing so wide a separation between growth and development is not clear. The use of the necessary biophysics and biochemistry as an introduction is no doubt logical, but in practice does seem to repel many students who will assimilate them comfortably if introduced in small quantities in their appropriate places.

Plant physiology, to Prof. Maximov's mind, is not an academic study. His book is expressly prepared for use in agricultural colleges as well as universities, and he regards agro-physiology as a good synonym for its title. The approach from the practical angle thus distinguishes the book from others with which it might be compared.

In adapting it to American conditions, the editors have introduced numerous modifications, presumably with the blessing of the author, though this is not stated. They have also added general references at the end of each chapter, but it is difficult to understand on what principle these were selected. Papers important enough to be dealt with in the text at some length are not always cited, while others are quoted which have no influence on the text and which would be difficult to get hold of and of limited value to a general student if obtained. Certain inconsistencies noted in the original book have been removed—notably in the chapter on respiration—and there seems every reason to anticipate a further long period of usefulness for it in its new form.

W. O. JAMES.

The Physiology of Plants. By WILLIAM SEIFRIZ. 9 × 6 in. Pp. 315 + vii with frontispiece and 95 figures in the text. New York: Wiley and Sons; London: Chapman and Hall. 1938. \$3.50.

There are now enough text-books of plant physiology available for students for any newcomer to need to justify itself by some degree of originality. It is therefore proper to say at once that this is quite unlike any other. Prof. Seifriz holds firmly that a good theory is more interesting than an alleged fact; and he seems to have a poor opinion of our data, calling them meagre and controversial. He therefore sets out to engage the student's enthusiasm by means of "theory supported by facts", and not by an encyclopaedia of facts alone. "The avoidance of finality in statement" follows as a natural corollary.

Two results of this attitude are particularly noticeable to the reader. The treatment is always brief, while physical and chemical principles believed to underlie physiological phenomena come in for relatively lengthy treatment. The colloidal state, osmosis and

acidity each receive a chapter with the minimum of complicating biological detail. On the other hand, the temptation offered by redox equilibria—which have shown some signs lately of becoming a vogue, as once *pH*—has been declined, and they are kept in their place.

Prof. Seifriz is also fond of the historical approach, and the fathers—and grandfathers—of the subject receive due mention and illustration, particular honour being done to Pfeffer. A wide scope must now be regarded as characteristic of Prof. Seifriz's writings, and the frontiers of physiology are here pushed well out on the ecological as well as on the physical and historical sides. It is doubtful whether this book will appeal much to the crammer, but in its chosen role of a stimulant to thought it will doubtless receive a good deal of attention.

W. O. JAMES.

A Textbook of General Botany. By R. M. HOLMAN and W. W. ROBBINS. 4th ed. 9 × 6 in. Pp. 664 + xv with 482 figures. New York: Wiley and Sons; London: Chapman and Hall. 1938. Price 20s.

Plant Form and Function. By F. E. FRITSCH and E. J. SALISBURY. 8½ × 5½ in. Pp. 668 + viii with 445 figures. London: Bell. 1938. Price 17s. 6d.

The first of these volumes keeps up to date a well-established text-book first published in 1924. The second is a lineal descendant of two previous books by Profs. Fritsch and Salisbury which it is intended to combine and supersede. The standard aimed at is about the same in both, viz. an adequate foundation course in general botany. We thus have two treatments of the subject of established reputation and assured future and it is interesting to compare them and to note their rather striking dissimilarities.

Plant Form and Function, much more than the *Textbook*, is for home use, being based on the theory that the subject is best taught in relation to what is at hand. There is for example a chapter of fourteen pages on the varieties of British Woodland, but that there is such a thing as tropical rain forest is not mentioned. Plant geography is limited to a consideration of the geographical components of the British flora, and a conspectus of British families is given in the final chapter. The method is not pushed to the illogical limit of excluding the Cycads from the Gymnosperms. In thinking of the theory on which the book is built one wonders whether moorland plants (for example) are really more available for study in London than the collections of Kew, or whether it is necessarily the function of a text-book to impart what can be most readily studied at first hand. In a general introduction the restriction seems a little artificial; and the unfortunate result of reading these two and some other elementary texts together was a keener perception of the poverty of our native range of plants.

In method of presentation there is also a wide divergence. The *Textbook* is "displayed" to the uttermost and pages employing six cases of type are frequent. Summaries and above all excellent illustrations are freely used, and a glossary and a copious index round off the whole. In *Plant Form and Function* the aim appears rather to have been the unfolding of a continuous narrative. Typographic emphasis is more sparingly employed and availability is entrusted to the indexing, which is very thorough. It may be said that this method makes general reading more attractive and escapes the suspicion of cramming which sometimes attaches to the other.

Ecology is given, as a considered policy, greater weight in *Plant Form and Function* than is usual. With an almost equal total of pages, fifty-eight are allotted to British plant associations as against six to North American associations in the *Textbook*. The balance is adjusted in describing the phyla, which, with the exception of the Algae, the former treats more briefly. The saving of space is largely at the expense of illustrations, and in another section, the chapter on the flower, I cannot help feeling that this has had the most unfortunate results.

Since the death of Prof. Holman the writing of the *Textbook* has to some extent gone into committee. The authors seem on the whole to have been well served by their experts and balance has not been sacrificed. *Plant Form and Function* has the advantage of greater

individuality, including a more decided point of view, and the corresponding drawbacks. Among these must be included a number of slips in the details of physiology.

Apart from such minor points the real interest in the simultaneous appearance of these two books is the contrast in their conceptions of how the subject should be taught. It has already been remarked in this journal (1935) that the method of the Californian text-book is familiar here and on the continent. The difference is therefore independent of geography and is a powerful stimulus towards a re-examination by the reader of his own ideas.

W. O. JAMES.

Plant Physiology, with Reference to the Green Plant. By E. C. MILLER, Ph.D. 2nd ed. 9 × 6 in. Pp. 1201 + xxxi with 39 figures in the text. New York and London: McGraw Hill. 1938. Price 45s.

No changes of aim or quality have been made in this new edition of Prof. Miller's text-book. The purpose of the first edition—to summarize the literature of plant physiology for "upper-classmen" with special emphasis on American and English sources—remains unaltered. An attempt at impartiality is maintained and the author has deliberately suppressed his own ideas, not feeling "qualified to speak with authority on a majority of the topics". Statements are not, in general, supported by appeals to evidence (since it needs a technical expert to know evidence when he sees it), but by appeals to Caesar. The Caesar index occupies 30 pages of small type.

The pages in the new volume exceed those of the old by 30 %. The increase in the amount of text is greater than this would suggest; to give more room, the questions following each chapter have been scrapped and a great deal has been put into small type. The illustrations have been kept sparse, perhaps for the same reason. At a rough estimate the number of references is now 6×10^3 and the number of words 6×10^6 . The fact that the mean allocation is a hundred words—including a citation in full—explains as much about the character of the text as a lengthy description.

The comprehensiveness that has been achieved is almost staggering. Stimulus physiology is still excluded, however, and it is a little surprising to find no reference to furanose sugars or respiratory "carriers". In a mood of depression the author suggests (p. 577) that the last fifty years have thrown little or no light upon photosynthesis. Fifty years ago the composition of chlorophyll was unknown; the major relations between rates and controlling agencies were misconceived; the existence of Blackman reactions was unrealized; and photochemistry was an unforged tool. The progress made lies in the fact that we are beginning to realize what it is we have to investigate.

In its chosen sphere this book is now quite without a rival. It performs the useful service of giving currency to a great deal of information that might otherwise remain "frozen" in obscure sources. It seems unlikely that any of those places where they study plant physiology will dare to be without it.

W. O. JAMES.

The Vegetation of South Africa. By R. S. ADAMSON, M.A., D.Sc., Professor of Botany in the University of Cape Town. London: British Empire Vegetation Committee. 1938. Obtainable from the Assistant Director, Royal Botanic Gardens, Kew, Surrey. 10s. 6d.

This is the first of a series of monographs to be published on the vegetation of the Empire. It contains a clear, concise description of the vegetation with an excellent account of the physiography, climates, geology and soils of South Africa, together with an account of the economic and sociological background. Prof. Adamson is familiar with all of the vegetational regions of the country, and is personally acquainted with every type described. His is the first comprehensive account to appear in this form, and he is to be congratulated on his masterly treatment of so vast and difficult a subject. Within the confines of a book of this size the author has achieved much to co-ordinate the scattered and incomplete information available, and to build a concise, lucid picture of the country.

The book contains 235 pages, divided into 11 chapters, with prefaces by Prof. A. G. Tansley and the author. The text is well illustrated by good photographs, and maps specially drawn for the purpose, together with numerous figures and charts. Chapter 1 deals with the physiography of the country and contains a general description of the Union, including boundaries, political divisions, physical features, vegetation, populations and races, agriculture and industries. The account of the physiography is concise and graphic. The concept of vegetation and its classification is introduced: five classes are recognized, *Bush*, *Forest*, *Savanna*, *Grassland*, *Semi-Desert and Desert*, and these are subdivided into twelve types. Chapter 2 gives a good account of the climate and weather, and is well illustrated by maps and charts. The principal features of the climates of the various types of vegetation are set out in a summarized form. Chapter 3 describes the geology and soils. In view of the scanty information available, the author has wisely desisted from an attempt at a complete classification of the soil types of the country, but has given instead a valuable account of the salient characters of the soils of the various types of vegetation. Chapter 4 deals with ecological factors in relation to distribution of the vegetation. The classification of types adopted and the boundaries of these as shown on the map do not agree completely with any of the arrangements put forward previously. Much has been gained in precision by the elimination of such general names as Karroo, High Veld, Thorn Veld, and others, as none of them is restricted to any one type of vegetation. It is rather unfortunate however that the term *Bush*, used as a name for one of the main classes of the vegetation, should be used again in association with subdivisions of quite a different class. This tends to confusion. Chapters 5-9 are devoted to the description of the five classes of vegetation. A general account of each class is given, including its distribution and a description of the habitat. This is followed by a more detailed account of the different types, including succession, development, modifications, and utilization. A general summary of the class is given at the end of each chapter. Chapter 10 is a short account of land utilization and natural products. Chapter 11 is devoted to general conclusions and prospects. The vegetation is considered in terms of larger biological-climatic-geographic units. The relations and affinities of the types composing them are discussed. Four groups are recognized, East Central African, Kalahari, Southern African, and Namib. Comparisons are drawn with other regions. Changes in climate are discussed, and the probability of recent change is considered in terms of changes in the vegetation. The author concludes with a consideration of the prospects of the country, pastoral, agricultural, and forest.

The book cannot fail to interest a very wide circle of botanists and geographers. It cannot fail to be for some years the standard reference to South African Vegetation and opens what we trust will be a long and profitable series of ecological monographs.

C. E. TIDMARSH.

THE EFFECT OF ETHYLENE ON THE RESPIRATION AND CARBOHYDRATE METABOLISM OF POTATOES

BY F. E. HUELIN¹ AND J. BARKER*Low Temperature Research Station, Cambridge*

(With 9 figures in the text)

INTRODUCTION

FOR many years remarkable responses of plant tissues to low concentrations of ethylene have been reported. The most precise of the earlier reports was that of Neljubow (1901, 1911), who showed that the etiolated epicotyl of the pea seedling was very sensitive to illuminating gas and demonstrated that ethylene was the effective constituent. He distinguished three definite degrees of response: (a) reduction of growth, (b) deviation of growth from vertical, (c) swelling of epicotyl with horizontal nutation and very slow growth. These degrees of response were also distinguished by Knight & Crocker (1913) who showed that they were produced by 0.1, 0.2 and 0.4 volumes of ethylene per million of air respectively.

The numerous papers which have been published on this subject report a great variety of effects on different plant tissues. Ethylene has been found to break the rest period of dormant tissues with the result that growth commences earlier (Vacha & Harvey, 1927).

Denny (1924) showed that ethylene accelerated the yellowing of citrus fruits and increased their respiration. He obtained an effect with 1 volume of ethylene per million of air, and a maximum effect at a concentration of 1 : 200,000. Since then ethylene has been found to accelerate ripening processes in many other fruits, including tomatoes (Rosa, 1925; Work, 1928), bananas (Wolfe, 1931), melons (Rosa, 1928), persimmons (Overholser, 1927), apples and pears (Allen, 1930).

Because of the unique effects of ethylene, more precise studies of its influence on various aspects of plant metabolism are needed. The most comprehensive study of the effect of ethylene on the respiration of plant tissues is that of Herklots (1928), who determined the effect of ethylene on the production of carbon dioxide of *Sequoia gigantea* twigs, cherry-laurel leaf, potato tubers, and ripening fruit. He reported three types of effect: (a) an initial rise followed by a gradual fall towards the control curve, e.g. in potatoes of very low sugar content; (b) an initial rise followed by a fall towards a value parallel to and above the control, e.g. in *Sequoia gigantea* twigs, very immature fruit; (c) an effect initially the same as (b) but followed by a second hump comparable to the normal respiratory hump of senescent tissues, e.g. ripening fruit, cherry-laurel leaf. Herklots obtained no effect with potatoes that had been sweetened at a low temperature. He regarded these effects as being due to "a decrease in the resistance of the protoplasm to substrate diffusion".

¹ Now on staff of Commonwealth Council for Scientific and Industrial Research.

In the investigation described in this paper a detailed study has been made of the effect of ethylene on the production of carbon dioxide and the sugar content of potato tubers.¹

EXPERIMENTAL METHODS

The Pettenkofer tube method was used to measure the respiration,² the air current being drawn from outside the building to avoid contamination with traces of volatiles.

In the analysis of sugar content the samples were frozen at -20°C . and ground while frozen. A weighed amount of the frozen tissue was extracted with boiling alcohol, the alcohol being subsequently removed by evaporation *in vacuo*. The water extract was cleared with basic lead acetate and excess lead removed with potassium oxalate. The reducing sugars were determined by the method of Shaffer and Hartmann. Total sugar was determined after boiling for 20 min. with 10% citric acid. The results were calculated in terms of invert sugar.

Effect of ethylene on potatoes stored at 15°C .

Preliminary experiments carried out with potatoes held at 15°C . revealed large variations in the response to ethylene of both the respiration and the sugar content (Huelin, 1931). It soon became apparent, however, that this variation was associated with the seasonal drift of the metabolism in storage and a series of experiments was accordingly planned to investigate in detail the change in the response to ethylene, with the progress of the storage period.

For this purpose a large stock of small potatoes (35–40 g. each) of the variety King Edward VII was harvested at the beginning of October 1931, and stored at 15°C . on 20 October. The first experiment was begun 5 days later, seven comparable samples, each of thirty-six potatoes, being used.³ After the respiration of each sample had been measured for 5 days, one sample was removed for sugar determination, five samples were transferred to 0.1% ethylene and the remaining sample was retained in pure air. The measurements of respiration were continued, and the samples in ethylene were analysed after 2, 4, 6, 10 and 14 days of the treatment. The other control sample was analysed 14 days after applying ethylene.

At intervals during the following 5 months, seven other similar experiments were carried out at 15°C . with samples from the stock stored at this temperature, and the results are all given in Figs. 1*a*, respiration, and 1*b*, sugar content.

Presentation of the complete records of respiration for every sample of the present series of experiments was not practicable, if all the results were to be plotted in one figure. It was accordingly decided to plot, for each experiment, only the final respiration values at the time the samples were frozen for analysis, together with the corresponding sugar values (Fig. 1); smoothed curves showing the drifts of respira-

¹ The influence of ethylene on the sprouting of potatoes has already been reported (Huelin, 1932).

² Throughout this paper the output of carbon dioxide is used as the measure of the respiration.

³ The potatoes started sprouting about a month later, and subsequently the sprouts of the main stock of potatoes had to be removed at fortnightly intervals.

tion and sugar content are also given, the ethylene curves being drawn as interrupted lines and the control curves as continuous lines (Fig. 1).

The range of variation encountered in the measurements of respiration is indicated by double lines in Fig. 4, in which the results are given for two experiments similar in type to the above, but carried out on a different stock of potatoes. As these

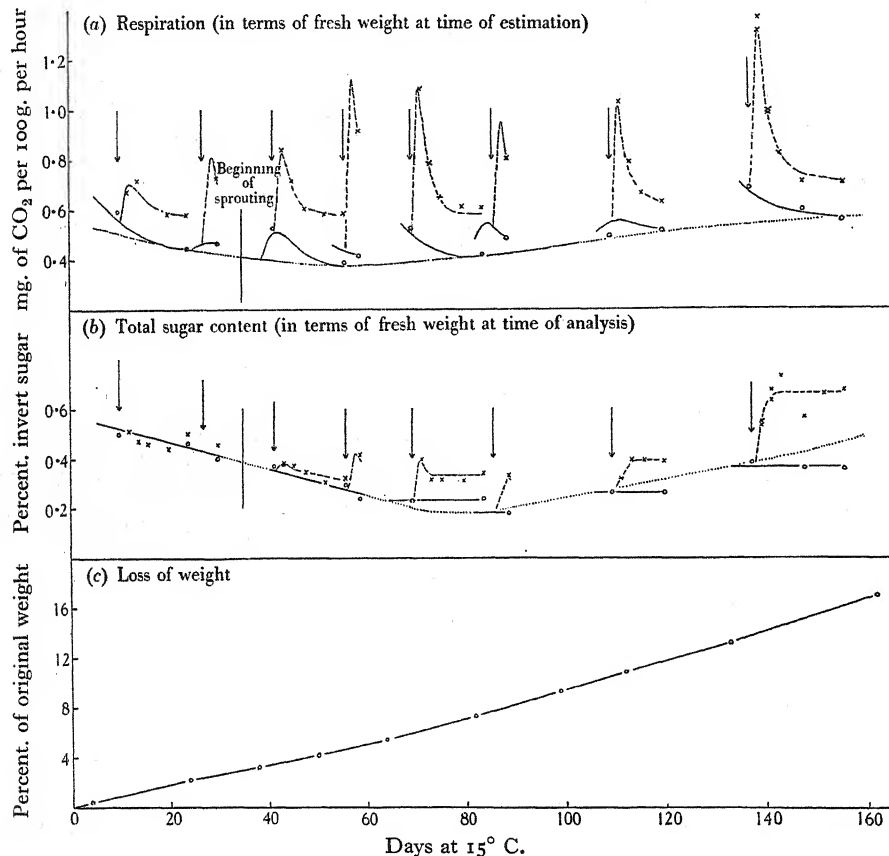


Fig. 1. Change in response to ethylene treatment during storage at 15°C. shown by English King Edward potatoes of low sugar content. Potatoes harvested 30 September 1931, and transferred from 10°C. to 15°C. on 20 October.

○ control values
× ethylene values
— control drift
--- ethylene drift
..... seasonal drift

Arrows mark times when samples were treated with ethylene.

experiments are typical it may be concluded that the use of smoothed curves introduces no large error.

From Fig. 1a it is evident that in each experiment the exposure to ethylene resulted in a rise in the respiration to a maximum after between 36 and 48 hr. in ethylene. The maximum respiration was followed by a prolonged fall to an "adjusted

state" in which the drift of respiration in ethylene appeared to be almost if not quite parallel with the control drift of respiration.¹

One measure of the magnitude of the effect of ethylene on the respiration is the percentage increase at the peak of the rise in ethylene as compared with the control value at the corresponding time. As judged by this measure the influence of ethylene on respiration increases during storage at 15° C. up to about 60 days; subsequently no consistent change occurred in the percentage increase of respiration.

In the first experiment (i.e. after only 10 days of storage at 15° C., Fig. 1*b*), no appreciable change in the sugar content resulted from exposure to ethylene. In the later experiments, ethylene caused an increase in sugar, the rise being greater the longer the period of previous storage. In some of the early experiments, an initial hump² in sugar content was followed by an "adjusted state" in which the drift of sugar was parallel with but above the control sugar; in the later experiments no hump was observed, the sugar rising directly to the "adjusted state".

Inspection of Fig. 1*a* shows that the drifts of respiration of the control samples for each experiment cannot be joined to form a smooth curve representing the course of respiration during storage at 15° C. In some of the experiments the control respiration drifted downwards from the beginning of the experiment; in other experiments the downward drift was preceded by a rise. Unpublished work (Barker, 1936) in which the respiration of the same sample, held continuously in a respiration jar, was measured throughout the storage life, has shown that the seasonal drift of respiration in storage at 15° C. actually follows a smooth curve, the drift being downwards early in storage and rising later in storage.

The variable behaviour of the control drifts of respiration (Fig. 1) was certainly not due to a direct effect of the growth of sprouts on respiration, since the samples had been desprouted immediately before the experiment commenced, and renewed growth was inappreciable for some 7 days. Nor does the vigorous development of sprouts and the handling required for their removal cause any permanent disturbance of the respiration provided the potatoes are handled carefully in desprouting (Fig. 2).

On the other hand, even gentle handling causes a small temporary increase in the respiration of turgid potatoes while with old flabby potatoes the increase is marked (Barker, 1935). The behaviour of the drifts of respiration of the controls (Fig. 1*a*) was thus probably due to the rather severe handling, the influence of which was not sufficiently appreciated at the time, during the desprouting and weighing at the commencement of each experiment. Accepting this explanation, it may be assumed that, as the handling effect gradually disappeared, the control curves tended finally to values which represented the true respiration of the undisturbed tissue. A dotted line has accordingly been drawn through the final value of each control curve to indicate the drift of respiration in undisturbed potatoes in storage at 15° C.

¹ In the experiments recorded in Fig. 1*a* the "adjusted state" lasted for at least 7 days. In other experiments, not recorded here, the "adjusted state" was found to continue for 14 days, when the experiment was terminated, without any indication of further fall in the respiration in ethylene towards the control.

² From these and other experiments not recorded here there seems no doubt that a hump in the sugar content actually occurs with some samples of potatoes.

Examination of the curves for total sugar content given in Fig. 1*b* shows that although the sugar values of the controls for the first four experiments can be joined to form a continuous curve representing the downward drift of sugar in early storage, subsequently the control curves for each experiment are horizontal although the pitches of the last three experiments indicate a general rise in the sugar with the progress of storage.

Unpublished work (Barker, 1936) shows that the downward drift of sugar in early storage at 15° C. is followed by a rise in sugar later in the season. In Fig. 1*b*, the curve (dotted line) representing drift of sugar content in storage has accordingly been arbitrarily drawn through the initial points of the last four control curves. The cause of the horizontal drift of sugar in the control samples in late storage is not

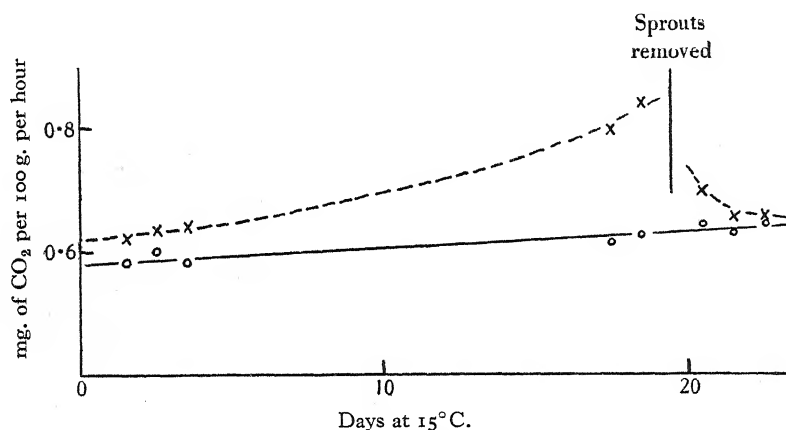


Fig. 2. Effect of sprouting on respiration of King Edward potatoes.

○ — sprouts removed weekly × — — — sprouts allowed to grow for 19 days

known; it may be suggested that change in sugar content during the later part of the storage period is largely governed by sprouting and that when this is reduced to a minimum,¹ the sugar content remains unchanged.

The loss of weight during the five months of storage was 16%. A considerable proportion of this loss was due to removal of sprouts.

It has already been noted that the magnitude of the response to ethylene treatment changed markedly with the progress of the storage period (Fig. 1*a, b*). In view of this apparent relation between the type of response to ethylene and the seasonal drift of the metabolism, it seemed essential to attempt to correct the control and ethylene curves for the different experiments so as to make the curves representative of potatoes whose metabolism had not been disturbed by the handling, etc., mentioned above.

¹ It was noticed that the potatoes in the respiration jars sprouted much more slowly than the remaining stock in storage. When samples were taken for measurements of respiration, they were desprouted more completely than was possible with a large stock, and subsequent sprouting was only apparent after about 10 days, when the sprouts were again removed.

In Fig. 3 the control respiration curves for each experiment have accordingly been adjusted to coincide with the assumed drift of respiration for undisturbed potatoes shown by the dotted line in Fig. 1*a* drawn through the final respiration values of each experiment. The values of the ethylene curves have then been altered in the same proportion as the control curves using the formula:

$$\frac{\text{Corrected ethylene value}}{\text{Uncorrected ethylene value}} = \frac{\text{Corrected control value}}{\text{Uncorrected control value}}$$

The sugar curves of Fig. 1*b* have been similarly corrected in Fig. 3*b*.

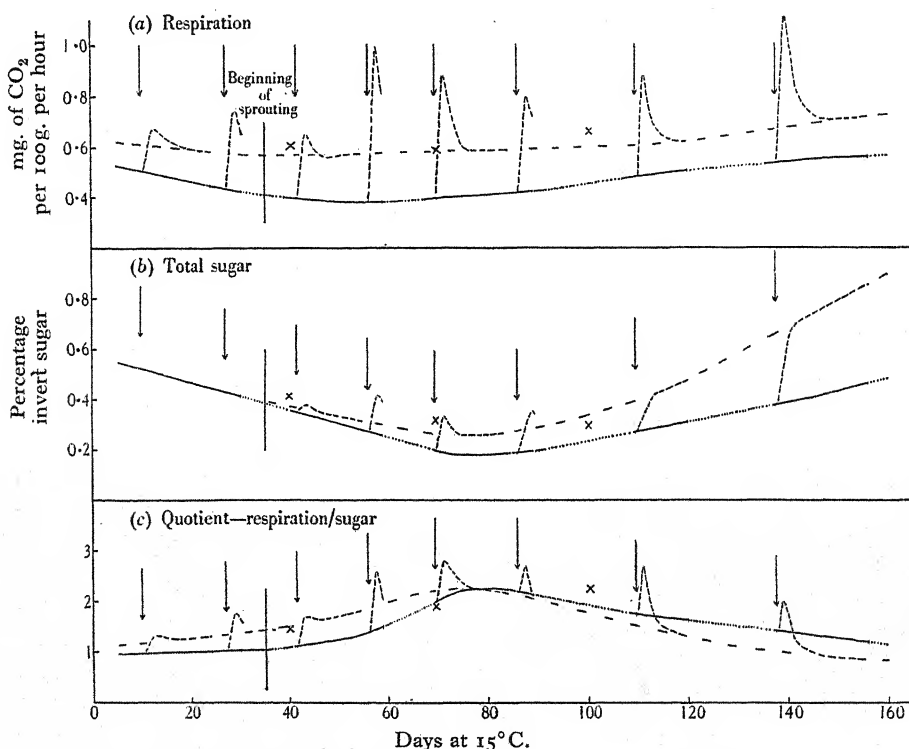


Fig. 3. Change in response to ethylene treatment during storage at 15°C. The curves of respiration and total sugar content are corrected from the corresponding curves of Fig. 1, as described in the text.

× values for samples kept continuously in ethylene — control drift
 - - - - - ethylene drift seasonal drift
 Arrows mark times when samples were treated with ethylene.

The procedure just described is clearly open to criticism; it should, however, be stated at once that no conclusion will be drawn from the corrected curves (Fig. 3) which is not evident, though less obviously so, from Fig. 1.

Inspection of the corrected respiration curves (Fig. 3*a*) reveals the same main features already described, i.e. in each experiment the hump or peak of the respiration response to ethylene was followed by a fall in respiration to an "adjusted state"

in which the drift in ethylene was roughly parallel with but above the control drift. It will be observed that a continuous curve (widely interrupted line) can be drawn through the "adjusted state" phases of each experiment suggesting that this curve may represent the respiration of samples held permanently in ethylene.

The adjusted sugar curves (Fig. 3*b*) show that, apart from the first experiment, ethylene caused an increase in sugar to a higher pitch which was then maintained; the magnitude of the increase in sugar was larger the longer the previous storage period. Just as in the case of the respiration drifts, a continuous curve (widely interrupted line) can be drawn through the "adjusted state" phases of the sugar drift in ethylene, again indicating that this curve may represent the drift of sugar in samples held continuously in ethylene.

A number of potatoes was kept continuously in 0.1% ethylene from the beginning of the first ethylene treatment, and samples were taken after 30, 60 and 90 days in ethylene for determination of respiration and sugar content. The values so obtained are plotted as crosses in Fig. 3*a, b* for comparison with the continuous ethylene curves. The respiration after 30 and 60 days in ethylene follows closely the continuous curve in Fig. 3*a*. The value obtained after 90 days lies above the curve, but there may have been a temporary increase of respiration due to handling. The values for sugar content plotted in Fig. 3*b* scatter about the continuous ethylene curve, but lie definitely above the control curve. This evidence thus supports the view that the widely interrupted curves in Fig. 3*a, b* actually represent the drifts of respiration and sugar in samples held permanently in ethylene.

In addition to the series of experiments just considered, tests were made with two other stocks of potatoes. With one stock (English King Edward VII, 1930) treatment with ethylene early in the storage period resulted in only a small rise in the respiration and no change of sugar content. Two months later exposure to ethylene gave a much greater increase of respiration and a considerable increase in sugar.

With the second stock (Teneriffe King Edward VII, 1931) exposure to ethylene gave no effect on the respiration during the first 35 days' storage at 15° C.; after this period, however, the response of the respiration (as measured by the method described on p. 88) increased with further storage (Table I).

Further experiments were made with samples of this Teneriffe stock which were held at 10° C. from the beginning of May and transferred to 15° C. on 16 July.

Table I. *Increase in response to ethylene after storage at 15° C.*
(*Teneriffe stock, stored 11 May 1931*)

Days at 15° C. before ethylene treatment	Percentage increase of respiration in ethylene
2	0
18	0
34	0
63	29
98	53

These samples were treated after 25 and 49 days' storage at 15° C., and the results are given in Fig. 4*a, b*. The curves of Fig. 4 have been adjusted similarly to those of Fig. 1 so as to make them representative of undisturbed potatoes, and the corrected curves are given in Fig. 5.

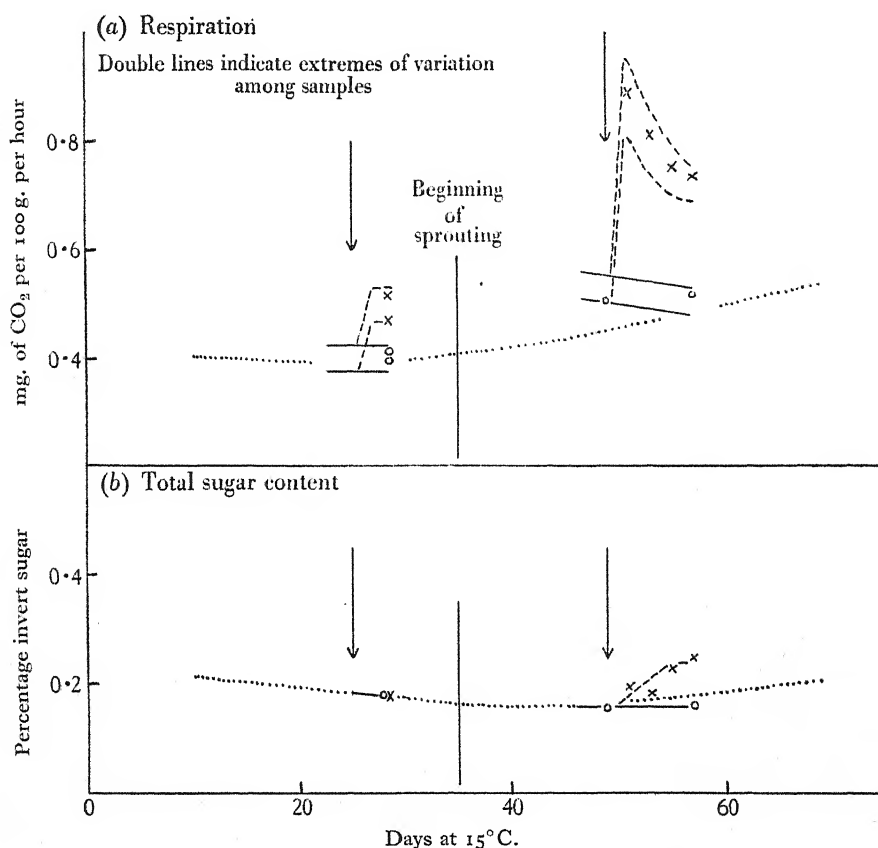


Fig. 4. Change in response to ethylene treatment during storage at 15° C. shown by Tenerife King Edward potatoes of low sugar content. Potatoes transferred from 10° C. to 15° C. 16 July 1931.

○ control values ——— control drift
 × ethylene values - - - - ethylene drift
 seasonal drift

Arrows mark times when samples were treated with ethylene.

These results thus confirm the data given in Fig. 1 in showing:

(a) That exposure to ethylene produces only a small effect on the respiration of potatoes treated shortly after harvesting (in the Tenerife stock ethylene had, in fact, no effect on the respiration for some weeks after potatoes were received), but that the effect increases in magnitude with continued storage.¹

¹ These observations suggest that ethylene might have no effect on the respiration of immature potatoes.

(b) That early in the storage period ethylene can cause an increase in the respiration without appreciable increase in the sugar content.

(c) That ethylene has no effect on sugar content early in the storage period, but that subsequently exposure to ethylene causes an increase in sugar content.

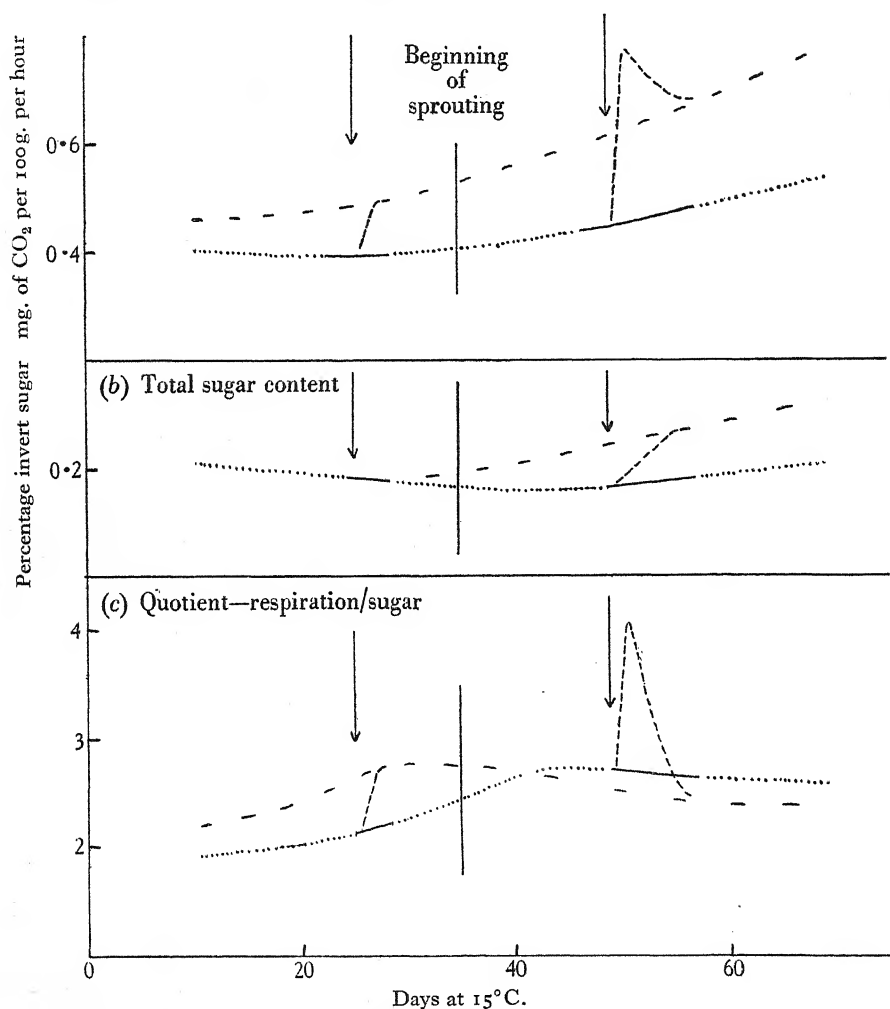


Fig. 5. Change in response to ethylene treatment during storage at 15°C. The curves of respiration and total sugar content are corrected from the corresponding curves of Fig. 4, as described in the text.

○ control values — control drift
 × ethylene values - - - ethylene drift
 seasonal drift

Arrows mark times when samples were treated with ethylene.

Effect of ethylene at 15°C. on potatoes sweetened at +1°C.

On 26 October 1931, twelve samples of English potatoes (thirty-six potatoes in each sample) were stored at +1°C. in order to increase their sugar content. After 85 days at +1°C. these samples were transferred to 15°C. and eight samples placed

in respiration jars for determination of respiration. After $3\frac{1}{2}$ days at 15°C ., one sample was removed for sugar determination and three samples were treated with 0.1% ethylene. Three days after applying ethylene, an ethylene and a control sample were removed for sugar determination; and further samples were removed 6 and 9 days after applying ethylene. One sample was treated with 0.1% ethylene after $9\frac{1}{2}$ days at 15°C . and removed for sugar determination 3 days later. The values for respiration and total sugar content are plotted in Fig. 6, and mean curves are drawn through these values. As already reported by various investigators both respiration and the sugar content fell rapidly at 15°C ., but for the first 10 days the values for respiration and total sugar content are unaffected by ethylene; subsequently they are higher than in the controls.

The effect of 0.1% ethylene was also determined after 23 and 38 days at 15°C . by using the four remaining samples. In the first of these two experiments, one sample contained a potato affected with blight and its curve of respiration was considerably higher than the other. The former sample was treated with ethylene, and its curve of respiration in ethylene was corrected by subtracting a figure equal to the difference between the two curves in air.

The curves of respiration in Fig. 6*a* are corrected in the same way as the curves of Fig. 1*a*, and the corrected curves are given in Fig. 7*a*. The curves for sugar content in Fig. 6*b* need no correction; with these sweet or partially sweet potatoes the amount of sprouting was very small and thus possibly insufficient to affect the total sugar content of the samples.

This experiment thus confirms the previous observation (Herklots, 1928) that ethylene has no effect on the respiration of potatoes of high sugar content. On the other hand, when the sugar has fallen to 2.0% ethylene produces a small response, and the magnitude of the response increases with further desweetening.

It is interesting to compare these results with those already described for potatoes held at 15°C . from shortly after harvesting (Figs. 1, 4 and Table I). Early in the storage period ethylene may be without effect (Table I) or only produce a small response (Fig. 1). With further storage, however, and the associated fall of sugar content (Fig. 1) the response increases.

It seems doubtful, however, whether the absence of, or small magnitude of, the response to ethylene in potatoes shortly after harvest is directly related to too high sugar content. Thus in the last experiment (Fig. 6) a response occurs in 2.0% sugar; in the Teneriffe potatoes in which no response was observed (Table I) the sugar content (unfortunately not determined) was probably not above 0.4%; moreover, in the experiments shown in Fig. 4 the response increased with further storage without any appreciable fall of sugar.

Reversibility of the ethylene effect

We have seen that with samples of potatoes which show an effect of ethylene on the respiration the response takes the form of a hump in the respiration, the peak of the curve being reached about 36 hr. after applying ethylene. If, however, the treatment with ethylene is terminated during the progress of this characteristic

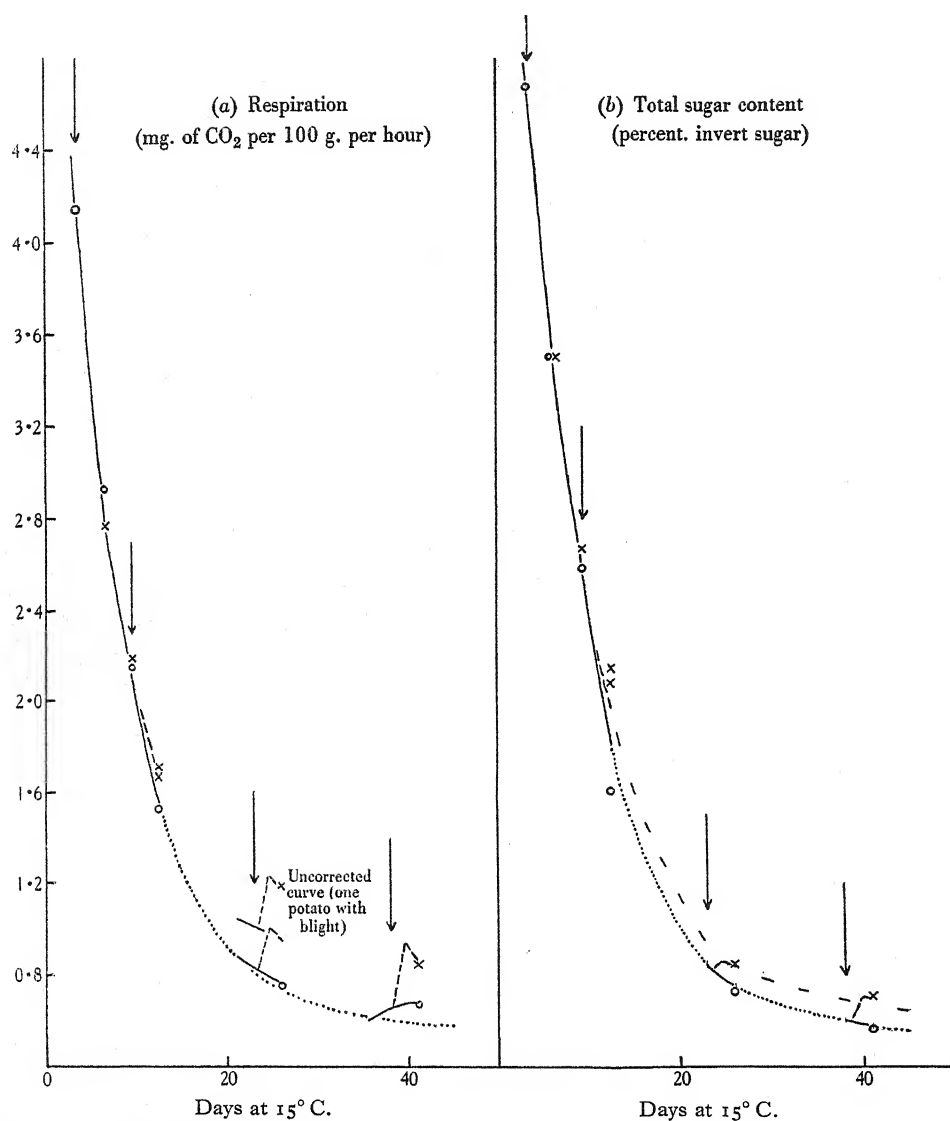


Fig. 6. Change in response to ethylene treatment during storage at 15°C . shown by English King Edward potatoes previously sweetened at $+1^\circ \text{C}$. Potatoes transferred to 15°C . 19 January 1932.

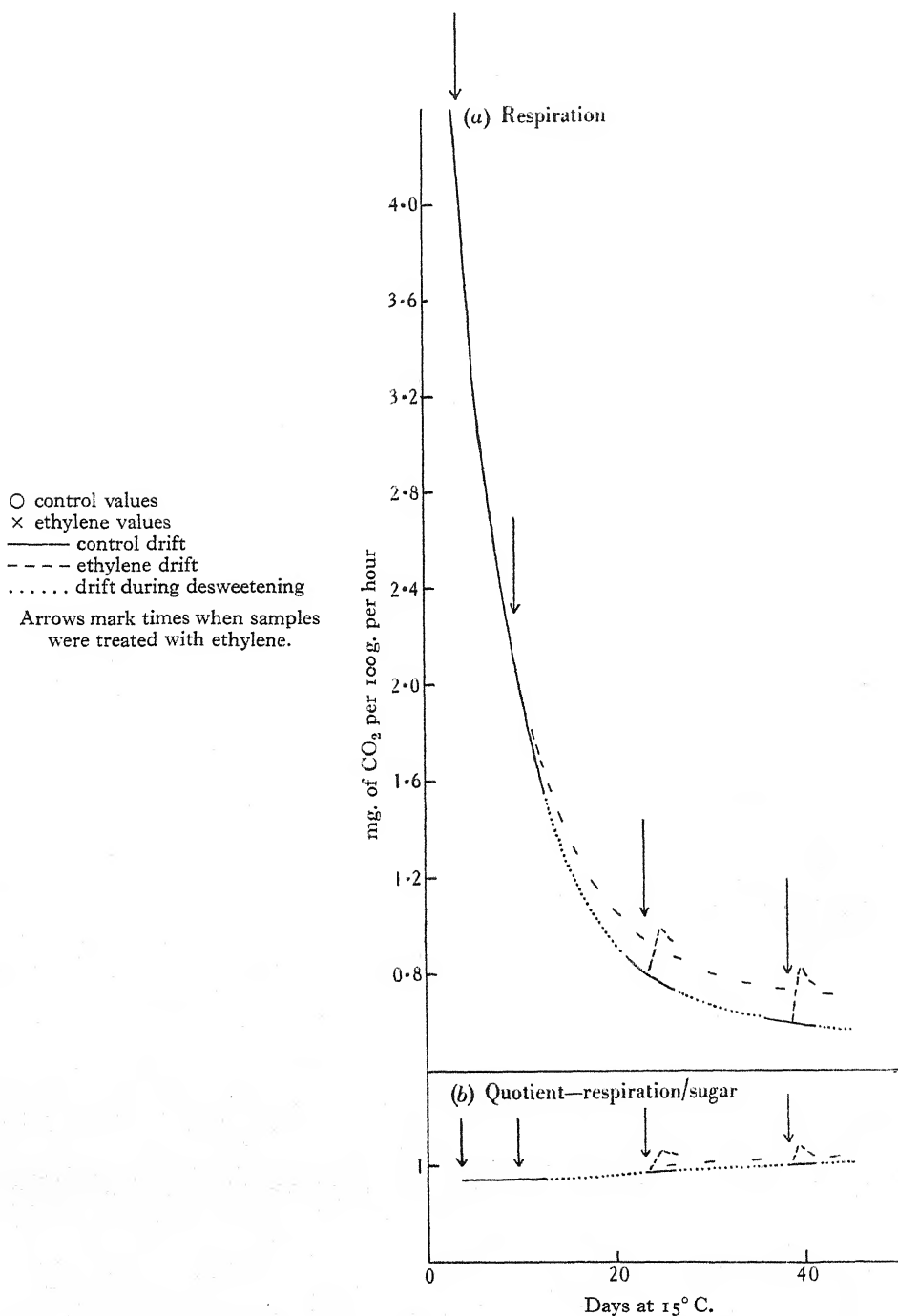


Fig. 7. Change in response to ethylene treatment during storage at 15°C. shown by sweetened potatoes. The curves of respiration are corrected from the corresponding curves of Fig. 6, as described in the text.

ethylene response, the potatoes slowly recover their ability to respond in this way when again treated with ethylene.

Three samples, each of thirty-six potatoes, were returned to air after being held in ethylene for 2, 6 and 14.5 days, respectively. Each sample was then divided into four samples of nine potatoes, and each of these sets of four samples was used to determine the effect of a second treatment with ethylene after either 7, 14, 21 or 28 days in air.

The results Fig. 8*a* show that recovery after 2 days' treatment with ethylene was rapid; after 14.5 days in ethylene, however (Fig. 8*c*), the second treatment after 7 days in air resulted in only a small hump in the respiration, and it was not until after 3 weeks that recovery was complete, i.e. the hump produced by the second treatment was as large as that produced by the first.

Fig. 8 also shows that the fall of the respiration in air after ethylene (widely dotted line from termination of first treatment) to the control level occupies some 20-30 days.

Although then the effect of ethylene on the respiration appears to be reversible, the full recovery, except after a very short exposure, may take 3 weeks or even longer.

Effect of different concentrations of ethylene

Two experiments were carried out to determine the effect of different concentrations of ethylene on the respiration of potatoes. The first experiment was carried out with potatoes from Teneriffe. Concentrations ranging from 0.0001 to 0.7% were used. Unfortunately the respiration of the control sample was upset by the leak of a small amount of ethylene into the pure air current. The percentage increase of the respiration was greater in magnitude with rising concentration up to 0.1% ethylene, but a higher concentration did not produce an appreciably greater effect (Fig. 9*a*).

The second experiment was carried out with English potatoes. Concentrations of ethylene ranging from 0.0001 to 0.1% were used and the maximum effect was obtained with a concentration of 0.01% (Fig. 9*b*).

It was found that after potatoes have been exposed for several days to 0.0001% ethylene, exposure to a higher concentration can produce a further hump in the curve of respiration. One sample of nine potatoes was treated with 0.1% ethylene, and the respiration increased to a maximum of 84% above the control. A similar sample was treated with 0.0001% ethylene, which produced a response of 14%. After 7½ days' exposure to 0.0001% ethylene, the second sample was treated with 0.1% ethylene, which produced a maximum increase in respiration of 111%.

Uptake of ethylene by potatoes

To estimate the amount of ethylene removed from an air current containing initially 0.1% ethylene, the current was divided into two equal parts each of rate 1 l. per hour. Half of the current passed over a sample of five potatoes, while the other half passed through an empty respiration jar. After absorption of carbon

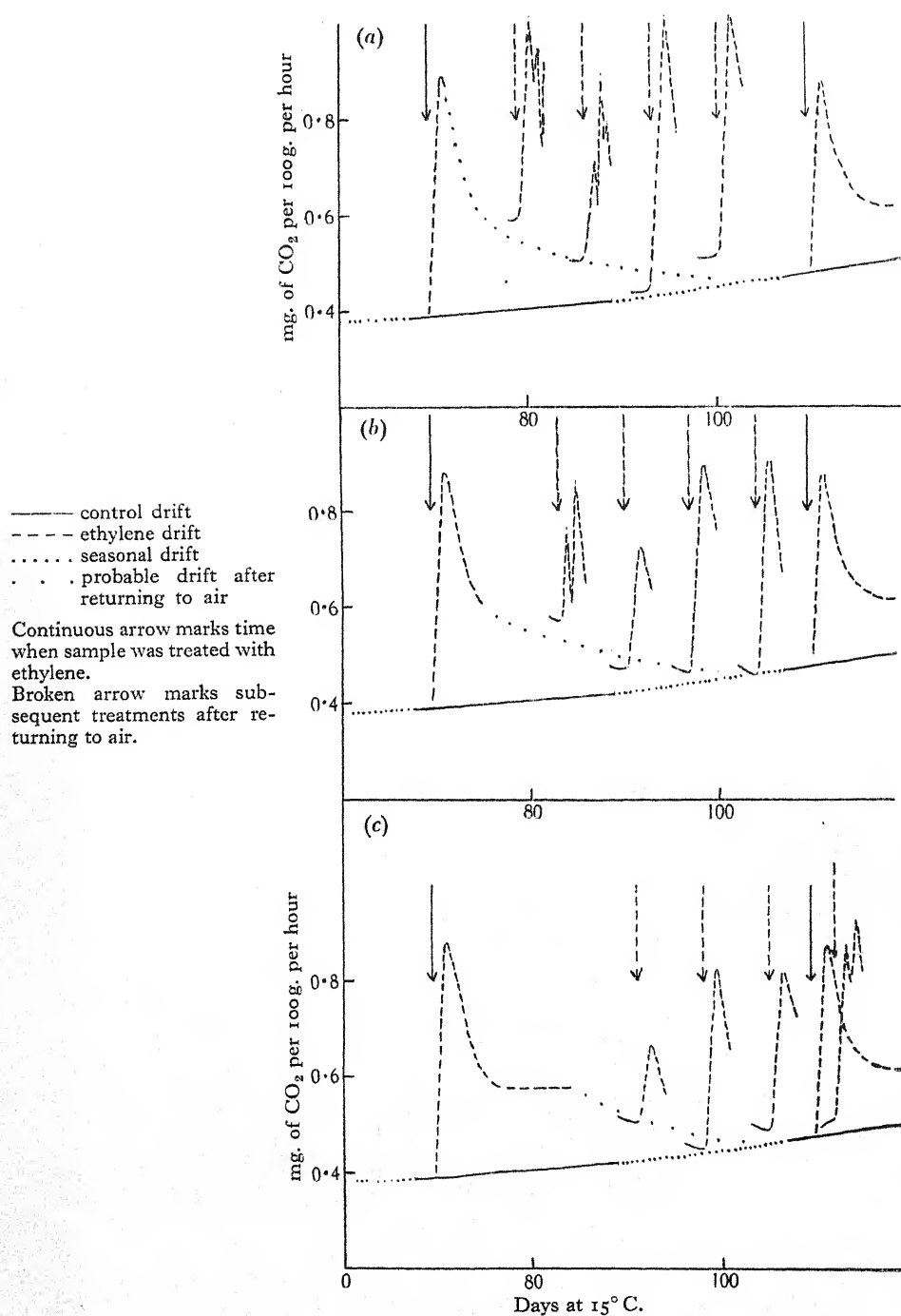


Fig. 8. "Recovery" of potatoes at 15°C. in air after first ethylene treatment as shown by response of respiration to subsequent ethylene stimulations.

dioxide in the Pettenkofer tubes, the ethylene was determined by passing each half of the air current over heated platinized asbestos and absorbing the carbon dioxide produced in two more Pettenkofer tubes. The ethylene absorbed by the potato sample was calculated from the difference between these two determinations. During 35 hr., while the respiration in ethylene was at a maximum, the amount of ethylene absorbed by the sample was 4.4 ml., hence not more than 23% of this excess carbon dioxide could arise from the oxidation of ethylene.

When lower concentrations of ethylene are used the percentage of excess carbon dioxide that could possibly arise from oxidation of ethylene is much smaller. In the

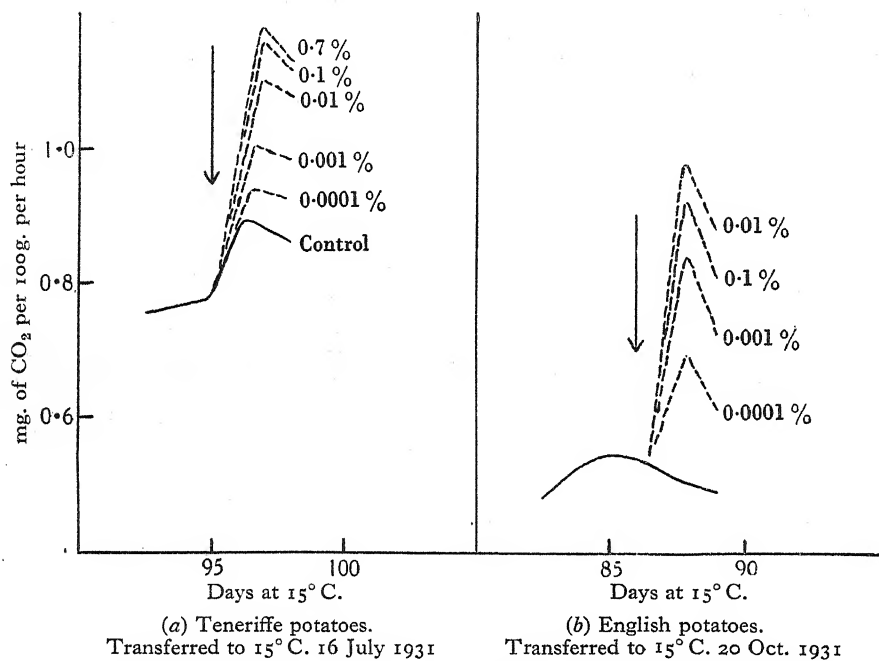


Fig. 9. Response of respiration to treatment with different concentrations of ethylene shown by King Edward potatoes at 15°C.

— control drift - - - - ethylene drift
Arrows mark time when samples were treated with ethylene.

experiment the results of which are given in Fig. 9*b*, the maximum rate of respiration in 0.01% ethylene was 3.1 ml. of carbon dioxide per hour. Ethylene in the air current was passing over the sample at a rate of 0.2 ml. per hour. Hence if all the ethylene was oxidized by the tissue, it could only give rise to 13% of the excess carbon dioxide.

Discussion

The observations set out above indicate the occurrence of two distinct effects of ethylene treatment on the metabolism of potato tubers, viz. (a) a rise in the respiratory efficiency (measured by the quotient respiration/total sugar—see below), and (b) a rise in the concentration of sugar, presumably as a result of the conversion of starch

to sugar. Exposure to ethylene shortly after the potatoes were harvested produced only the first effect; the respiration increased without any measurable change of sugar content (Figs. 1, 4). Later in the storage period, however, exposure to ethylene caused increases of both respiration and sugar content (Figs. 1, 4). In the latter cases then the rise of respiration might be accounted for by an increase in the concentration of respirable substrate, directly induced by the increase of measurable sugar.

Several investigations have shown that the respiration of potatoes is markedly influenced by changes of the sugar content, and increases of respiration, similar in magnitude to those caused by ethylene, have been produced when the sugar content was increased, either by storage at low temperatures, or by exposure to low concentrations of hydrogen cyanide; in fact, up to about 1.0% total sugar content the respiration at 15° C. has been found to be nearly proportional to the sugar (Hanes & Barker, 1931; Barker, 1933).

In order to ascertain the extent to which the changes of respiration observed in ethylene could be attributed to alterations in the sugar content, values for the respiratory efficiency, i.e. quotients for respiration/total sugar,¹ have been calculated from the various sets of data plotted in Figs. 3*a*, *b*, 5*a*, *b*, 6*b* and 7*a*; the quotients are plotted in Figs. 3*c*, 5*c* and 7*b*, smoothed curves, continuous lines for the control samples and interrupted lines for those in ethylene, being drawn through the points for each experiment.

Examination of Fig. 3*c* reveals a well-marked drift of the respiration/sugar quotients of the control samples, the values rising during the early storage period and falling again later.

Exposure to ethylene produced a quick rise in the respiration/sugar quotient, and this rise was followed by a fall to an "adjusted state"; in the first half of the storage period, when the respiration/sugar quotient in the controls was rising, the pitch of the "adjusted state" of the quotient in ethylene was higher than the control value; in the second half of the storage period, when the respiration/sugar quotient of the controls was drifting downwards, the quotient in ethylene fell below that of the controls. Moreover, a curve (widely interrupted line, Fig. 3*c*) could be drawn through the "adjusted states" of the separate experiments; this curve may represent the drift of the respiration/sugar quotient of potatoes held in ethylene throughout the storage period.

The data in Figs. 3 and 5 suggest that the response to ethylene may be dual in nature and consist of:

(*a*) An "adjustment phase" of 5–10 days in which the respiration and the respiratory efficiency (respiration/sugar quotient) rise immediately to the *adjusted* level (phase *b*), or, more usually, first rise above this level and then fall to it, giving the characteristic hump or peak in the respiration and respiratory efficiency; the sugar content either remains unchanged or also increases to an *adjusted* level.

¹ Although under certain conditions the respiration of potatoes was more closely correlated with the concentration of sucrose than with that of the hexoses (Barker, 1936), for the data under consideration it was immaterial which sugar fraction was used as the measure of the respirable substrate, since little change occurred in the proportion of sucrose to hexose; total sugar content was adopted as avoiding implications as to the nature of the respirable sugar.

(b) An "adjusted phase" of prolonged but undefined duration, in which the respiration, sugar content and respiratory efficiency in ethylene drift nearly parallel with, but at different pitches from, the drifts of these functions in the controls. The relations of the pitches of the "adjusted phases" for respiration, sugar and respiratory efficiency to the control pitch at different stages in the storage period are clearly shown in Fig. 3.

The rise of the respiration in ethylene was thus never wholly accounted for by the increase in the sugar content. Although the increase of sugar could (owing to direct effect in increasing the concentration of the respirable substrate) account for a part, and in late storage ("adjusted phase") for the whole of the increased respiration, there was always an increase of the respiratory efficiency (temporary only in late storage).

In seeking to analyse the response to ethylene we have then to account for (a) the increase of sugar, (b) the increase of respiratory efficiency in the "adjustment phase", and (c) the observation that the respiratory-efficiency in the "adjusted phase" was above the control pitch in early storage but later fell below the control drift.

Critical evidence of the mechanism of the action of ethylene on plant metabolism is still lacking, but its influence certainly appears to be general rather than specific. Thus in accelerating the ripening of certain fruits, ethylene must increase the activity of numerous enzyme systems; such an influence can scarcely be specific to each of the various enzymes concerned.

The effect of ethylene on the respiration and sugar content of potatoes must then be ascribed to some general aspect of the protoplasmic control of metabolic rate; e.g. to an effect on the grade of the "organization-resistance" as conceived by Blackman & Parija (1928). Thus these authors pictured the resistance or hindrance to reaction as achieved either by spacial separation of the reactants by impermeable protoplasmic membranes, or by adsorption or combination of reactants by stabilized components of the protoplasm; changes in the grade of the organization-resistance could occur either spontaneously (e.g. during senescence) or by experimental treatment.

The most precise evidence of the mechanism of action of ethylene on the respiratory metabolism of potatoes appears to be the observation, previously made by Herklots (1928) and confirmed here, that ethylene does not affect the respiration of potatoes whose sugar content has been increased to a high pitch by exposure to low temperatures; such high sugar potatoes, however, regained the power to respond to ethylene if the sugar content was reduced by storage at higher temperatures. This observation clearly suggests that the effect of ethylene on the respiration is concerned with the supply of the substrate to the respiratory centres. Where the rate of supply of respirable substrate is high, as in high sugar potatoes, ethylene has no effect on respiration; where the supply is low, due to low sugar content, ethylene may increase respiration, possibly through an effect on rates of diffusion. It will be noted that this line of evidence does not support either the hypothesis that ethylene activates the respiratory enzymes or that it initiates an abnormal type of

respiration;¹ such effects would equally be expected to occur in potatoes high in sugar.

The change in the type of response to ethylene with progress of the seasonal drift of metabolism in storage also merits further consideration. Treatment with ethylene shortly after the potatoes were harvested was shown above not to alter the sugar content; as the storage period extended, however, the increase of sugar produced by ethylene became progressively larger. With one stock of potatoes no change in the respiration resulted from treatment shortly after the samples were harvested² and in all stocks tested the response of both the respiration and the respiratory efficiency increased in magnitude in the early part of the storage period. Here then may be evidence that the organization-resistance is high when the potatoes are dug, and that the increasing response to ethylene with continued storage shown by the sugar content and the respiration is due to a progressive weakening of the resistance, ethylene exerting more effect the weaker the resistance. A spontaneous weakening of the organization-resistance with the progress of senescence has already been postulated for apples (Blackman and Parija, 1928).

With many tissues, e.g. fruits and leaves, ethylene is known to accelerate the progress of senescence or at least of certain senescent processes. In its effect on the pitch of the respiratory efficiency in the "adjusted phase" ethylene appears to exert a senescent influence on the metabolism of potatoes. Thus the data shown in Fig. 3c indicate that ethylene affected the "adjusted phase" so that the respiratory efficiency was changed to a pitch not reached in the control samples until some weeks later; in early storage when the control respiratory efficiency was rising the "adjusted phase" was above the control pitch; in late storage when the respiratory efficiency of the controls was falling the "adjusted phase" fell below the control pitch.

During the second half of the storage period the changes of the sugar content of the untreated samples (Fig. 3b) may be ascribed to a progressive weakening in the resistance to hydrolysis caused by senescent changes in the organization-resistance. The effect of ethylene in increasing the sugar content may thus be due to a senescent influence, exposure to ethylene weakening the resistance to hydrolysis.

Finally, reference should be made to the differences which become apparent when the effect of ethylene on the respiration of the potato is compared with its influence on various fruits. It has been shown above that the continuous exposure of potatoes to ethylene results in an increased rate of respiration as compared with untreated samples; when the ethylene treatment is terminated the respiration slowly falls to the control level (Fig. 8). Exposure to ethylene also causes a marked increase in the respiration of apples, pears and bananas which are in the pre-climacteric stage; the effect is, however, irreversible,³ the respiration showing no tendency to return to the control pitch when the treatment is terminated (Kidd & West, 1932; Hansen & Hartman, 1937; and Gane, 1935, 1937).

¹ No determinations were made of the respiratory quotient during an ethylene response. Such determinations might be valuable in detecting an abnormal type of respiration.

² The lack of, or small, response of freshly dug potatoes seems unlikely to be due to the rather higher sugar content at this stage than later in storage (see p. 94).

³ Except possibly with immature apples, treated shortly after picking (Kidd & West, 1934).

Moreover, ethylene is without effect on the respiration of apples, pears and bananas in which the climacteric rise has begun.

The explanation of these observations appears to be that while potatoes do not produce appreciable quantities of ethylene, fruits such as apples, pears and bananas, after a certain stage in senescence, evolve ethylene in such quantities that their metabolism is affected by the ethylene they are themselves producing. In the pre-climacteric stage the production of ethylene would appear to be so small that, except under conditions where the escape of the ethylene is prevented (Kidd & West, 1934), the concentration in the cells is too low to be effective; exposure to ethylene, however, causes a rapid increase in the respiration—the climacteric phenomenon—and in this stage the fruit is found to be evolving appreciable quantities of ethylene (Gane, 1935). There is thus considerable justification for the suggestion that the irreversible nature of the climacteric rise in respiration is due to the continuous auto-stimulation due to the fruit's own production of ethylene; the observation that treatment with ethylene is ineffective in the climacteric stage also finds immediate explanation on the same basis.

SUMMARY

The effect of ethylene on the respiration and sugar content of King Edward potatoes at 15° C. has been determined after varying periods of storage at this temperature.

The characteristic effect was an increase in the respiration to a maximum during the first 2 days after exposure to ethylene followed by a fall in the respiration to an adjusted state above the control value.

Increases in respiration were obtained in a concentration of one part per million of ethylene. The response increased with rising concentration up to one part per thousand.

Ethylene had only a small or no effect on the respiration of potatoes shortly after harvesting, but the effect increased in magnitude with continued storage.

Early in the storage period ethylene caused an increase in respiration without an appreciable increase in sugar content.

Later in the storage period ethylene caused increases both in the respiration and the sugar content.

Ethylene had no effect on potatoes of high sugar content, which had been sweetened at +1° C.

Potatoes returned to air after an ethylene treatment slowly recover their ability to respond to further treatment.

It is suggested that ethylene causes a lowering of the "organization-resistance", resulting in changes in the "respiratory efficiency" and the starch-sugar equilibrium which are similar to those occurring in senescence.

We wish to thank Dr F. Kidd for his interest in the investigation.

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THE CONTROLLING INFLUENCE OF CARBON DIOXIDE

VI. THE EFFECT OF THE TENSION OF OXYGEN AND OF CARBON DIOXIDE IN THE ATMOSPHERE UPON THE COURSE OF CHEMICAL CHANGE IN STORED APPLES

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(With 8 figures in the text)

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INTRODUCTION

THE purpose of the work, the results of which are dealt with in this paper, was to study the influence of oxygen and of carbon dioxide upon the progressive changes in chemical composition that take place during the storage of mature Bramley's Seedling apples after gathering. Two temperatures of storage were used, 1 and 5° C., because it was hoped that the results might afford some clue as to why functional breakdown and death of the tissue occur prematurely when these apples are stored at 1° C., although they remain healthy when stored at 5° C. It is known that Bramley's Seedling apples will not tolerate long exposures to temperatures lower than about 3° C. in air and that carbon dioxide accelerates the onset of functional breakdown at temperatures below this (Kidd & West, 1930).

METHODS, MATERIALS AND ERRORS

The methods employed for sampling and for the control of the atmospheric composition and of temperature have been described in detail elsewhere (Kidd & West, 1933). For the present experiments ninety-four homologized samples were prepared. Ten were used for analysis immediately after gathering (28 September

1933, initial samples), and six stored in each of the following atmospheres at 5° C. and also at 1° C.:

Varying oxygen; carbon dioxide less than 0.5 %	Varying carbon dioxide; oxygen 10 %
A. 2.5 % oxygen	C. Less than 0.5 % carbon dioxide
B. 5.0 % "	E. " " 5.0 % " "
C. 10.0 % "	F. " " 10.0 % " "
D. 21.0 % "	G. " " 15.0 % " "

In each case one of the six samples was removed from storage for analysis after 26, 69, 113, 138, 173 and 219 days. Samples consisted of twenty-five fruits (average weight per fruit = 129 g.). These samples were prepared for analysis by freezing,

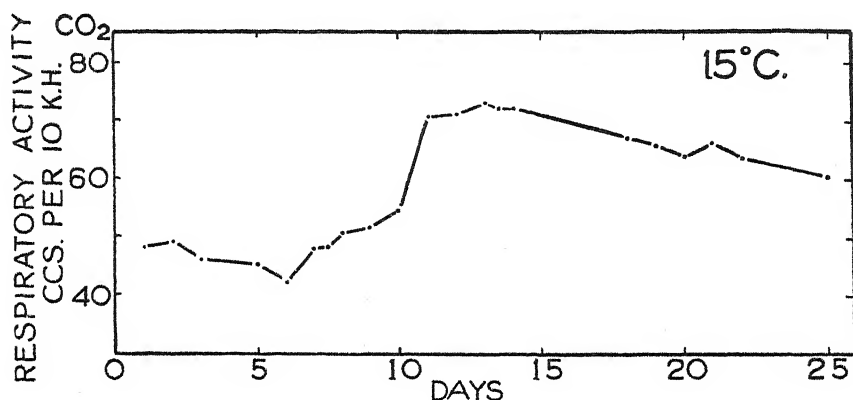


Fig. 1. Respiratory activity in air of Bramley's Seedling apples used in the present experiments.

grinding and mixing as described previously (Onslow *et al.* 1931). Some hours elapsed between removal of the fruit from store and placing it at -20° C. for freezing, but this interval was approximately the same in all cases (about 20 hr.). The following fractions were determined: glucose, fructose, cane sugar, starch, malic acid, alcohol-insoluble residue. The methods used are described in detail elsewhere (*Spec. Rep. Food Invest. Board*, 1939) and in outline in the Appendix. It is worth emphasizing that the fractions determined are ones of which a definite measure is obtained by the methods used. While there is little doubt that these fractions consist mainly of the substances stated, they may include other substances.

It has been found by experiments at different temperatures and in different atmospheres that the behaviour of stored apples is greatly affected by the exact stage of maturity at gathering, and elsewhere we have used respiratory activity as a criterion of the stage of maturity (Kidd & West, 1937). In these experiments, therefore, the respiratory activity of the material was determined at 15° C. The results are shown in Fig. 1, and they indicate that the apples were gathered at what may be regarded as the optimum stage of maturity for long-period storage, namely, about a week before the climacteric rise in respiratory activity at 15° C. begins.

The results of the chemical analyses are expressed throughout as percentage of

original fresh weight. The determined fractions constituted 12.98% of the fresh weight of the fruit when gathered, and the undetermined fraction in the alcohol extract was of the order of 1-2%. The average difference between replicate determinations made on the same sample was very much smaller than that between determinations made on replicate samples, and hence only a single set of analyses was as a rule carried out on each sample. The primary data are given in full in Table I. The standard error of determination and sampling from the ten initial samples, as regards the different components estimated, is as follows:

Table I

	Mean values for ten initial samples	Standard deviation of single sample
1. Glucose	1.075	0.102
2. Fructose	5.647	0.091
3. Sucrose	2.798	0.091
4. Total sugar	9.520	0.096
5. Starch	0.639	0.032
6. Acid	1.108	0.035
7. Alcohol-insoluble residue	2.353	0.046
8. "Residue", i.e. alcohol-insoluble fraction less starch	1.714	0.033
9. *Total "fructose"	7.046	0.100
10. †Total "glucose"	3.113	0.076
11. Total sugar + starch	10.159	0.102
12. Sum of all determined fractions	12.980	0.132

* Free fructose + $\frac{1}{2}$ sucrose.

† Starch + free glucose + $\frac{1}{2}$ sucrose.

It appears therefore that the standard error of the initial values, that is, of the mean of ten samples, is relatively small.

LOSS OF ACID, TOTAL SUGAR + STARCH AND "RESIDUE"

The primary data of the analyses are given in the Appendix. Rates of loss from straight lines of best fit have been calculated (Table II) for the fractions (1) total sugar + starch, (2) acid and (3) "residue".

Taking the deviations from the straight lines of best fit (of the six observations subsequent to the common initials) for the whole set of experiments and allowing 5 degrees of freedom for each set of six points, standard deviations were calculated from the formula

$$\frac{\sqrt{\sum (y - Y)^2}}{35},$$

where y represents a point and Y a corresponding point on the fitted line. When these values of standard deviation are compared with the standard deviation values from the ten initial samples, the ratios of the variances are:

	1° C.	5° C.
"Residue"	1.09	0.94
Acid	0.94	1.30
Total sugar + starch	2.80	3.50

$Z = 0.52$ (5% level) corresponds to a ratio 2.82.

Table II. Rates of loss of component fractions (g./100 g. original fresh weight/100 days)

	Less than 0.5 % carbon dioxide					10 % oxygen			
	2.5 % oxygen	5 % oxygen	10 % oxygen	21 % oxygen	0 % carbon dioxide	5 % carbon dioxide	10 % carbon dioxide	15 % carbon dioxide	
1° C.									
Total sugar + starch:									
Rate of loss	-0.261	-0.258	-0.324	-0.296	-0.324	-0.172	-0.145	-0.288	
Mean loss rate	-0.345	-0.298	-0.551	-0.578	-0.551	-0.278	-0.519	-0.560	
Average rate of loss	-0.289	-0.270	-0.368	-0.421	-0.368	-0.197	-0.290	-0.352	
Acid	-0.143	-0.133	-0.141	-0.183	-0.141	-0.154	-0.173	-0.237	
Residue (less starch)	-0.103	-0.091	-0.098	-0.118	-0.198	-0.079	-0.059	-0.017	
Total of above	-0.505	-0.483	-0.570	-0.597	-0.570	-0.405	-0.377	-0.542	
5° C.									
Total sugar + starch:									
Rate of loss	-0.165	-0.372	-0.332	-0.402	-0.332	-0.229	-0.210	-0.357	
Mean loss rate	+0.037	-0.294	-0.369	-0.720	-0.369	-0.108	-0.150	-0.308	
Average rate of loss	-0.135	-0.363	-0.433	-0.673	-0.433	-0.246	-0.207	-0.302	
Acid	-0.167	-0.185	-0.207	-0.228	-0.207	-0.176	-0.166	-0.188	
Residue (less starch)	-0.118	-0.134	-0.132	-0.140	-0.132	-0.078	-0.072	-0.085	
Total of above	-0.449	-0.691	-0.827	-1.023	-0.827	-0.484	-0.448	-0.630	
S.E. of rates of loss:	Total sugar + starch	0.0381	(19:1) level of significance	0.0999			
	Acid	0.0128	"	"	"	0.0355	
	Residue (less starch)	0.0125	"	"	"	0.0346	
	Total	0.0491	"	"	"	0.0363	

This can only mean that there is a substantial variation in the rate of loss of total sugar and starch with time or that the variance increases with time. On general grounds the first alternative seems the more likely.

In view of the above we have calculated for the total sugars + starch fraction mean loss rates (sum of losses divided by sum of times) and average rates of loss (sum of loss/time ratios divided by six), working from the mean of the ten initial samples. These values appear in Table II for comparison with the values for rates of loss determined by the straight line of the best fit.

All three components, namely, sugars, acid and alcohol-insoluble "residue", show significant losses during storage, and it therefore seems probable that they are all, directly or indirectly, sources of carbon for the carbon dioxide of respiration.

At 5° C. the determined loss of each component increases with the tension of oxygen over the range 2.5–21 %.

At 1° C. the values obtained indicate a minimum loss of all components in 5 % oxygen, the loss in 2.5 % being slightly greater than that in 5 % oxygen in all cases. The difference between the results in 2.5 and in 5 % oxygen is not large enough to be statistically significant.

Increasing carbon dioxide tension appears to reduce the loss of the fraction sugars + starch up to tensions in the neighbourhood of 10 %. The effect of carbon dioxide upon acid loss is similar to its effect upon sugar loss at 5° C., but at 1° C. the result appears to be reversed, carbon dioxide causing an increase in acid loss. Statistically, however, the increased loss of acid with 5 % carbon dioxide at 1° C. as compared with no carbon dioxide, is not significant, and we suspect that there may in fact be a reduction in rate of loss with increasing carbon dioxide up to about 5 %. "Residue" loss appears to be significantly reduced by carbon dioxide up to about 10 % at 5° C. At 1° C. "residue" loss is reduced by carbon dioxide up to 15 %.

The results set out in Table II indicate fairly clearly that the concentration of oxygen at 5° C. has a far greater effect upon loss of sugar than upon loss of acid or loss of residue. Carbon dioxide, on the other hand, affects sugar loss and loss of residue about equally and loss of acid to a less extent.

Another interesting fact brought out in this table is that increased carbon dioxide tension has a far greater effect in reducing "residue" losses than have low oxygen tensions even down to 2.5 % oxygen.

While we have treated the form of the quantity-time relation for acid and for "residue" as linear, the relationship which has generally been found to hold in other researches is not strictly so. The rate of acid loss has usually been found to diminish with time except under conditions of low temperature when breakdown occurs and the rate of acid loss increases concomitantly (Haynes, 1925). The rate of loss of "residue" is often, especially in the case of fruit gathered immature, more rapid at first, only becoming approximately linear after an initial period of varying duration.

In Fig. 2 are plotted the quantity-time relationships for the average of all results at 5° C. and of all results of 1° C. for acid and for "residue" respectively. On the average after about 100 days at 1° C. breakdown begins together with an increase in

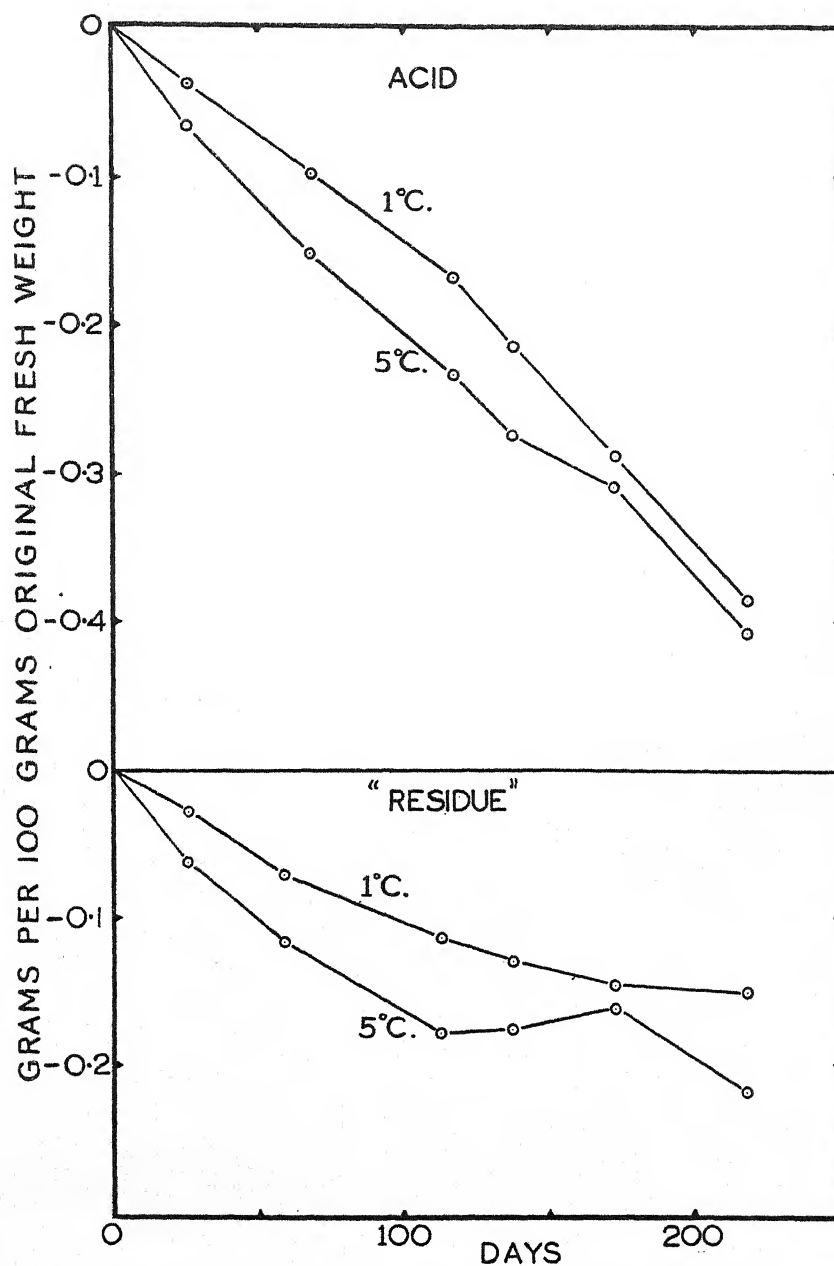


Fig. 2. Quantity-time plotting of average results for all gas-mixtures at each temperature (losses).

the rate of loss of acid. It seems likely that the anomalous result noted above, that carbon dioxide increases the rate of acid loss, may be due to the effect of carbon dioxide upon breakdown at low temperatures. We have shown elsewhere that carbon dioxide increases the amount of breakdown occurring and shortens the interval before its appearance (Kidd & West, 1930).

The results for "residue" in Fig. 2 show that on the average the rate of loss diminishes with time. At 5° C. there may even be a net gain in the "residue" fraction late in the storage life.

In Fig. 3 the average results for total sugars + starch are treated in the same way as those for acid and "residue" above. We have already shown that the variation of the observations about straight lines of best fit was so much greater than that of the ten initial samples about their means as to suggest that the rate of loss in this case was definitely fluctuating with time. At 1° C. the rate of loss in the first period is higher than that during any other period in each of the seven experiments. At 5° C. there seems on the average to be a period of reduced rate of loss round about the middle of the storage period. As will be seen in the following analysis of the course of events as regards the "total fructose" fraction and the "total glucose" fraction, it appears probable that these irregularities are due to traffic between these fractions and the undetermined fraction.

STARCH AND SUCROSE HYDROLYSIS

The present data give no information on the question of the influence of oxygen and carbon dioxide on the rate of disappearance of starch, since in all cases starch had completely or almost completely disappeared by the time the first set of observations were made, i.e. after 26 days.

Sucrose usually increases during the disappearance of starch, reaches a maximum during this process, and then itself disappears in approximate agreement with the formula

$$\log (\text{sucrose}) = kt.$$

The present authors have found in investigations on storage in air that

$$\log (\text{starch} + \text{sucrose}) = kt$$

gives a good agreement with the most critical sets of observations they have made. An examination of the mean losses on the basis of the second of the above equations reveals no significant difference in the rate of loss of cane sugar as between 2.5 and 21% oxygen. Carbon dioxide, on the other hand, appears to increase the rate of loss, but not to any marked extent. This is in agreement with previous findings (Kidd & West, 1927, 1933).

TOTAL "FRUCTOSE" FRACTION AND TOTAL "GLUCOSE" FRACTION

In studying the changes that occur in the reducing fractions the position is complicated by the fact that these changes are without doubt at least the summation of losses by respiratory consumption and gains from hydrolysis of starch and cane

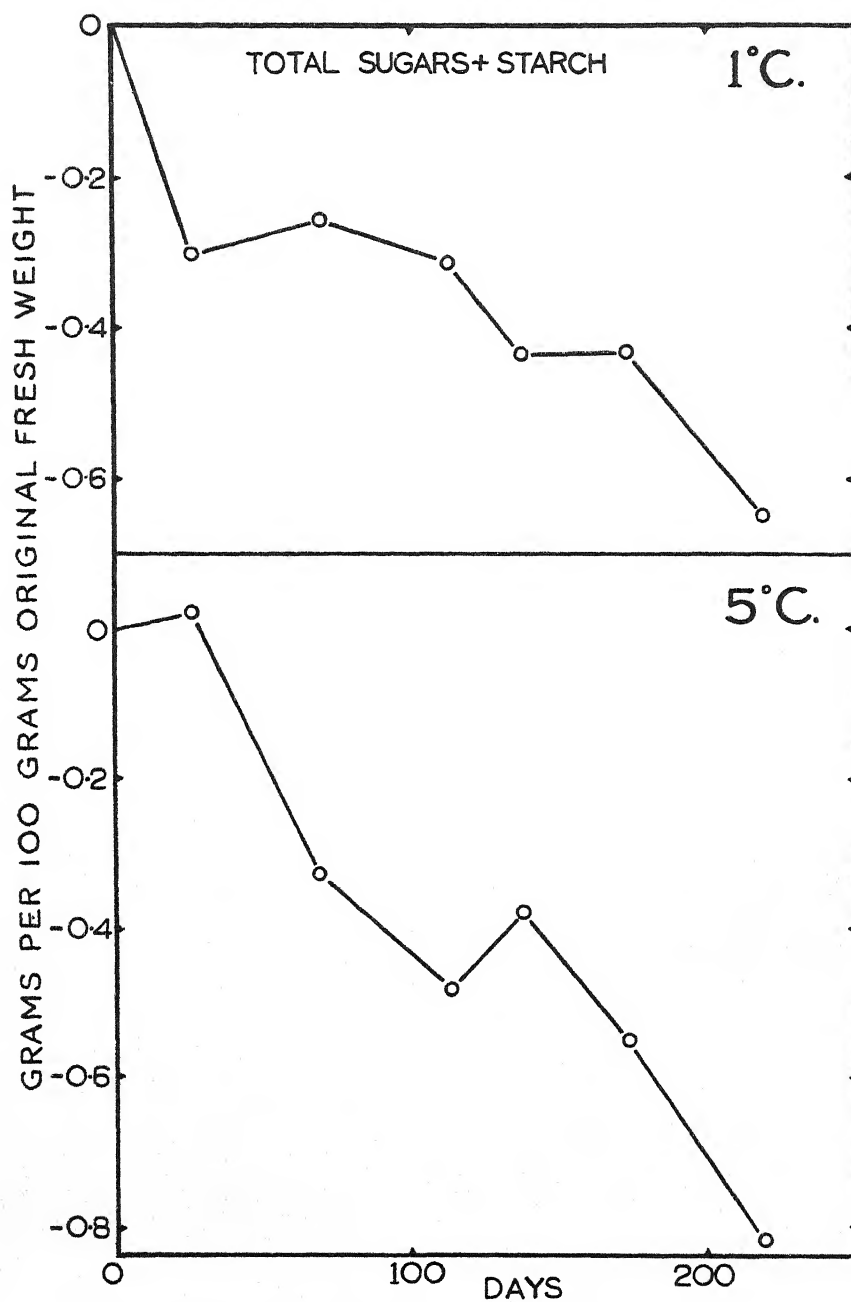


Fig. 3. Quantity-time plotting of average results for all gas-mixtures at each temperature (losses).

sugar. We adopt, therefore, the procedure of Onslow *et al.* (1931) and plot against time (*a*) the sum of starch (as hexose), half-sucrose (as hexose) and free glucose, and (*b*) the sum of half-sucrose (as hexose) and free fructose. In this way the curves will be the resultant only of losses due to respiratory drain, of any loss or gain due to interconversion of glucose and fructose and of interchange with the residue or the undetermined fraction.

Employing this method we shall deal elsewhere with data which establish clearly that in apples after gathering there is a rapid conversion of "glucose" units to "fructose" units (during the hydrolysis of starch when the fruit is kept in air). This conversion under normal conditions of atmosphere and temperature appears to be of the order of 50% of the starch hydrolysed.

The present data as a whole yield the results shown in Table III for the first period of 26 days during which starch hydrolysis is completed.

Table III. *Average of the seven observations of the two gas series after 26 days' storage*

	Total fructose	Total glucose	Total sugar
1° C.	+0.051	-0.360	-0.309
5° C.	+0.297	-0.273	+0.023

Loss of total sugars and starch at the mean rate in 26 days is 0.081 at 1° C. and 0.091 at 5° C.

The values in Table III indicate that at 5° C. glucose units have been converted to the extent of about 50% to fructose units during the period of starch hydrolysis. At 1° C. the state of affairs is possibly somewhat different. The gain of "fructose" appears to be significantly too small and the loss of total sugar + starch significantly too large, suggesting a transformation of some of the glucose during this first period of storage into a substance that escapes estimation, i.e. a non-reducing, alcohol-soluble substance.

If we now consider the period following starch hydrolysis and assume that the "fructose" and "glucose" fractions are not subject to interconversion or to interchange with residue or undetermined fractions, we should expect to find the scatter of the observations about straight lines fitted to the six points in this period (i.e. omitting the initial observation) to be normal and we should also expect to find these lines cutting the zero lines at values equal but of opposite sign; and these values should be a measure of the conversion of glucose units to fructose units which has taken place during starch hydrolysis. The results in air at 5° C. treated in this way are set out in Fig. 4. Those of the average of all results at 5° C. and of the average of all results at 1° C. appear in Fig. 5, while in Figs 6 and 7 the separate plottings for each experiment are given.

The loss of carbon as carbon dioxide is known from numerous experiments to be subject to no quick changes of rate at 1 and 5° C., and to follow an approximately linear course in quantity-time plotting. For example, in another series of experiments, not dealt with in full here, the results shown in Fig. 8 were obtained.

Three points can now be made:

In the first place, from the average slopes (Fig. 5) of the best fit straight lines at 5 and at 1° C. we obtain the following ratios 5°/1° C.:

"Glucose"	6.58
"Fructose"	1.65
Total	3.44

The normal ratio for loss of carbon in respiration is about 1.5 (Kidd & West, 1935).

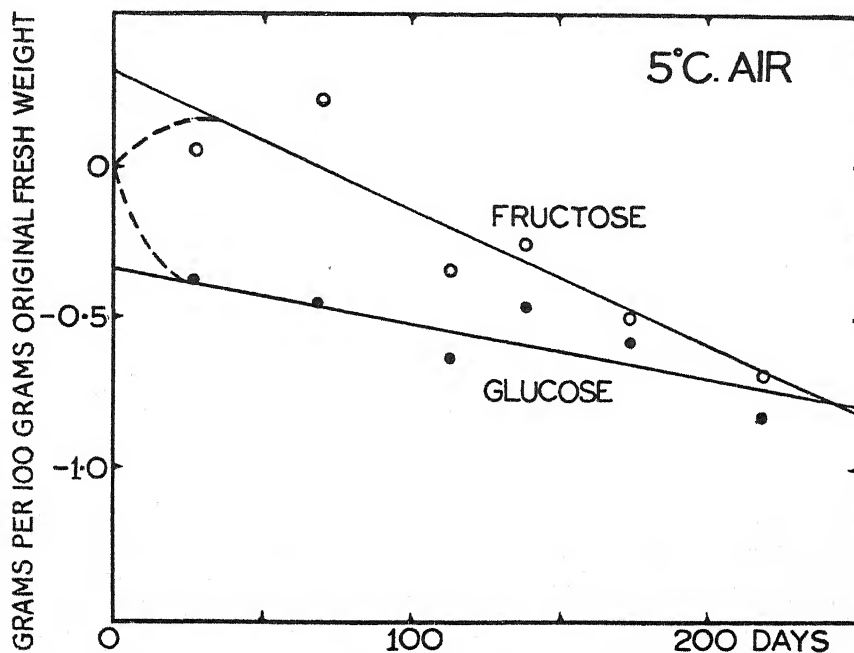


Fig. 4. Quantity-time plotting of values for total "fructose" and total "glucose" in air, with best fit straight lines (see text).

In the second place the scatter of the points about straight lines is not normal. Applying the same test as that used above in the case of total sugars + starch, to the deviations from best straight lines through the six points (omitting the first) for total "glucose" and total "fructose" for each of the seven conditions at each temperature, the ratios of the variances so obtained to those obtained from the initial ten samples are:

	1° C.	5° C.
Total "glucose"	2.3	2.4
Total "fructose"	2.4	5.4

$Z=0.52$ (5% level) corresponds to ratio 2.82.

The variance is thus very probably greater in all cases than is to be expected, but almost certainly so in the case of "fructose" at 5° C. It must therefore be taken as

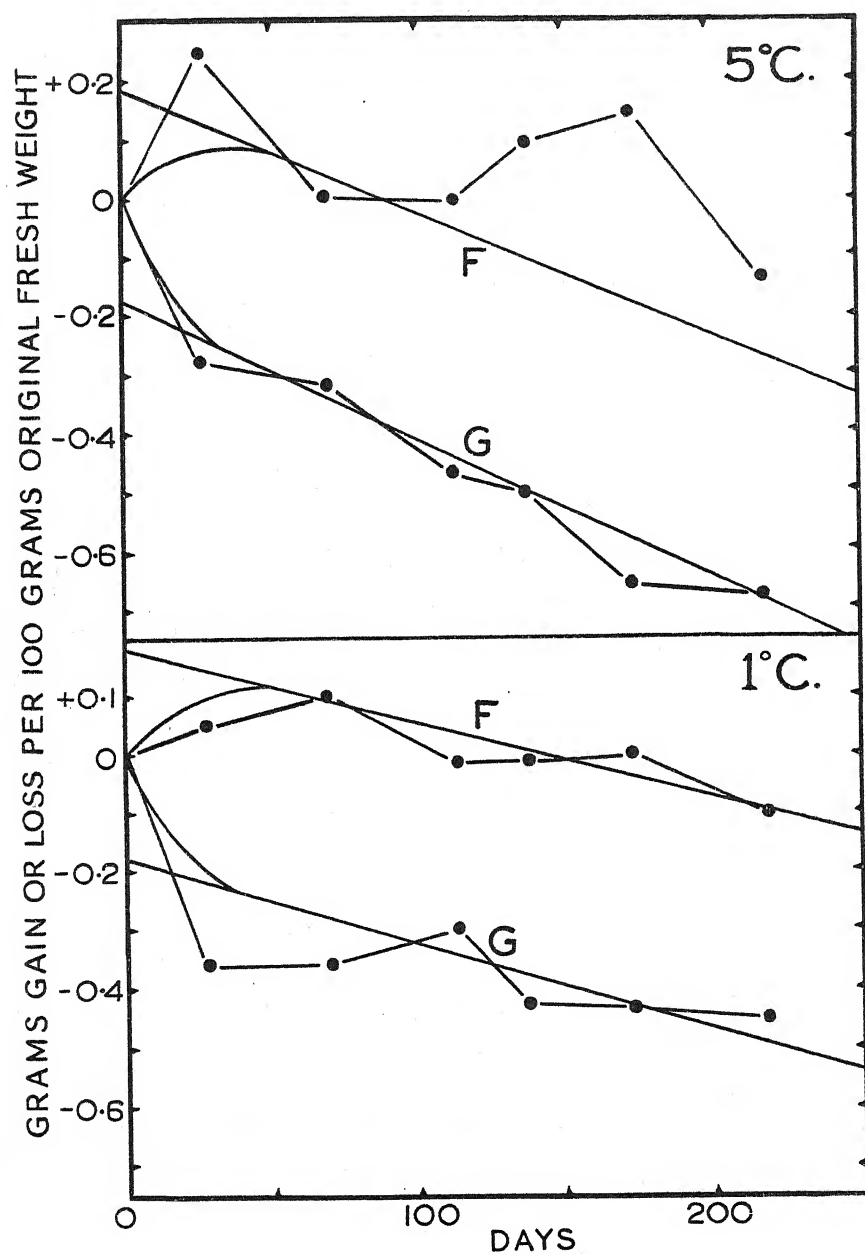


Fig. 5. Quantity-time plottings of average results for all gas-mixtures of total "fructose" and total "glucose" with arbitrary construction lines. F=total "fructose", G=total "glucose".

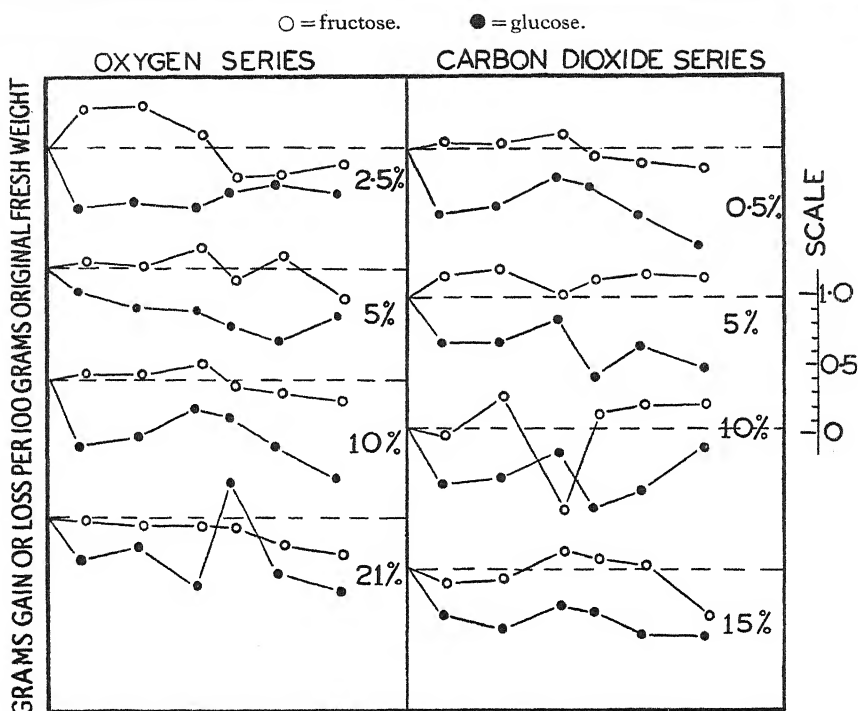


Fig. 6. Quantity-time plotting of total "fructose" and total "glucose" at 1°C.

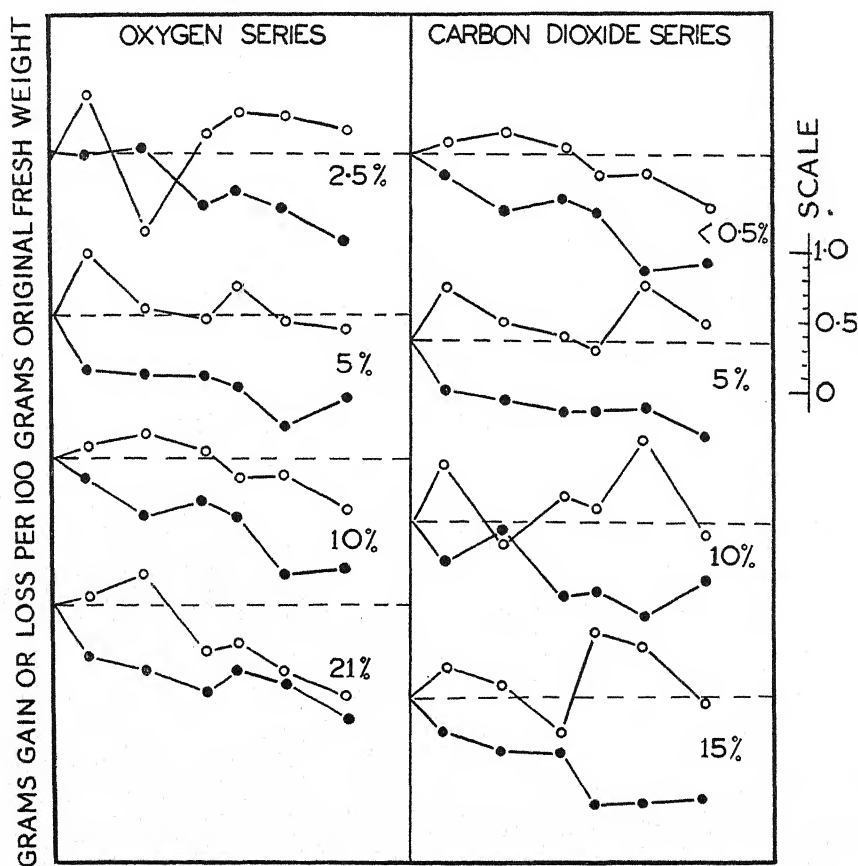


Fig. 7. Quantity-time plottings of total "fructose" and total "glucose" at 5°C.

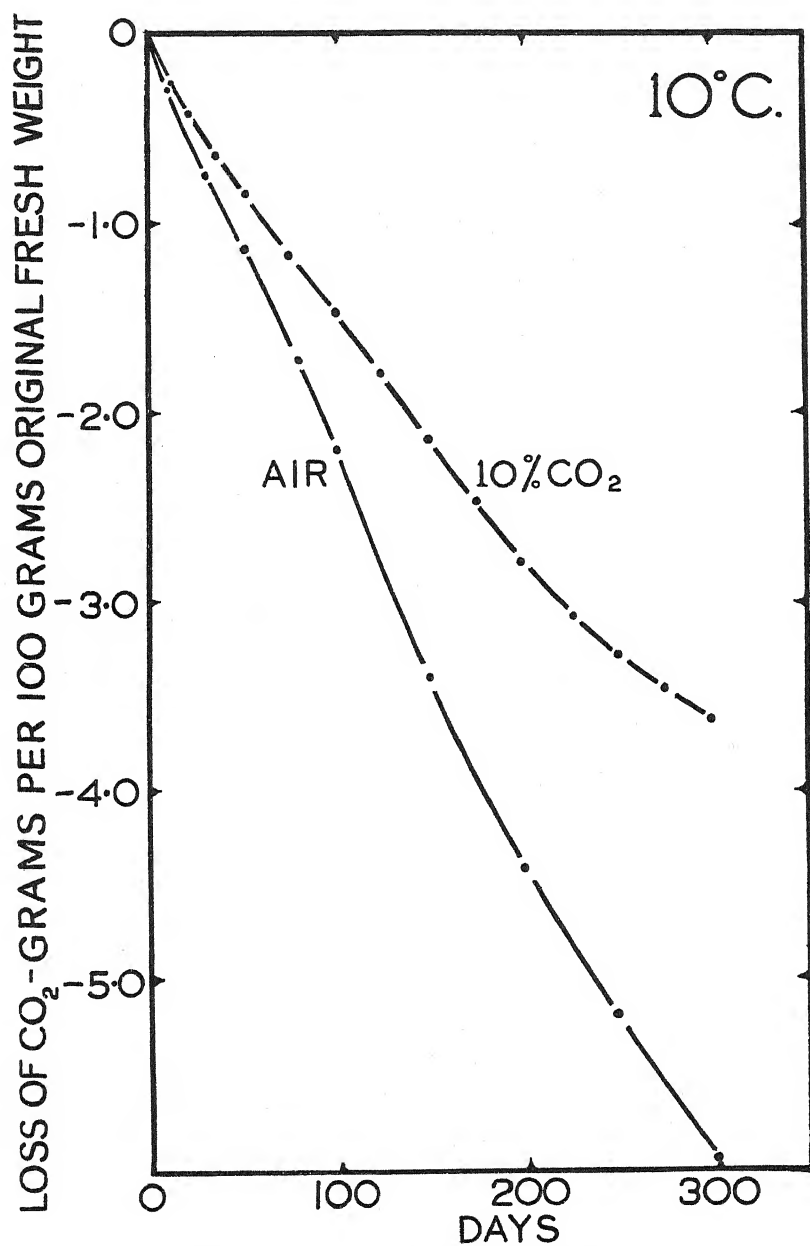


Fig. 8. Illustrating approximately linear quantity-time relationship of carbon loss as CO₂.

certain that at 5° C. at any rate the change in total "fructose" subsequent to starch hydrolysis is not approximately linear, and hence we must conclude either that the respiratory drain on "fructose" is not constant or that there are fluctuations during loss or gain of fructose units by conversion or by some other kind of transformation. Examination of the individual curves of Figs. 6 and 7 suggests that there may be an influx of "fructose" in the later part of the storage period, and that this phenomenon is most pronounced in the carbon dioxide series at 5° C. It seems clear from the results of the "residue" measurements that if such an influx occurs it must be from the undetermined fraction.

Thirdly, the average values for the intercepts of these best straight lines at zero time are:

	1° C.	5° C.
"Glucose"	-0.320	-0.189
"Fructose"	+0.133	+0.222

At 5° C. these values agree with expectation in so far that the "glucose" and "fructose" values are approximately equal but of opposite sign. The 1° C. values, however, again suggest that in the earlier part of the storage period at this temperature there may be a transformation of glucose to some substance escaping estimation. The individual results in Fig. 6 indicate that this early excess disappearance of glucose occurs in every case at 1° C. except that of the lowest oxygen percentage, i.e. 2.5%. Other results in our possession have indicated that at 1° C. the conversion of "glucose" to "fructose" during starch transformation goes through an intermediate stage, a fructosan being formed which escapes estimation but which later yields "fructose". There is obviously a possibility that this may have occurred in the present experiments except in the case of 2.5% oxygen.

The construction lines in Fig. 5 are inserted to represent the course of events as it is suggested they would appear in the absence of complications due to interchanges with the undetermined fraction. The ratios 5° C./1° C. of the slopes are approximately 1.5 and the conversion is approximately 50% of the starch.

It would seem therefore that little can be said with any certainty about the effects of temperature and of the concentration of carbon dioxide and oxygen on the course of carbohydrate change until the fraction at present undetermined can be brought into the picture. Thus, although inspection of Fig. 7 leads at first sight to the conclusion that at 5° C. lowering the oxygen concentration does not affect the rate of glucose loss, but cuts down progressively the respiratory drain on fructose, we cannot be sure of this because of the uncertainty as to whether fructose "influx" may be progressively increased by diminishing the tension of oxygen.

PHYSICAL CHANGES IN CONSISTENCY OF THE FLESH

The rate at which the apples softened at 3° C. was studied, using the same sampling technique and the same series of "gas"-mixtures. The measurements obtained were the pressures in pounds required to drive a plunger 7 mm. in diameter

a definite distance (7 mm.) into the fruit. For each value given four measurements were made on twenty fruits.

Table IV shows that carbon dioxide has a marked effect in retarding the softening. The influence of oxygen tensions upon softening is slight in comparison, but softening is definitely slower in 2.5% oxygen than in air. A comparison of these results with those obtained for "residue" losses (Table II) confirms the view generally held that softening is due to a decrease in the amount of alcohol-insoluble cell-wall material. It is interesting that oxygen and carbon dioxide tensions affect the hydrolysis of this material while they do not influence the rate of cane sugar hydrolysis.

Table IV. Penetrometer readings in pounds pressure

Days	Oxygen series (<0.5% CO ₂)				Carbon dioxide series (10% O ₂)			
	2.5%	5%	10%	21% O ₂	>0.5%	5%	10%	15% CO ₂
0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
27	10.1	10.9	10.1	9.1	10.1	11.2	11.0	11.0
69	11.1	9.3	6.7	8.5	6.7	11.9	10.5	11.2
104	8.5	6.2	6.0	6.8	6.0	11.0	11.0	11.0
139	7.0	6.6	6.2	6.4	6.2	10.2	10.1	11.3
174	7.1	7.1	6.1	6.2	6.1	9.1	11.0	10.4
230	6.5	5.7	5.4	5.9	5.4	8.1	10.2	11.2
Means	8.6	8.0	7.2	7.6	7.2	10.2	10.5	10.9

SUMMARY AND CONCLUSIONS

1. Each of the three main fractions of the mature apple, namely, (a) the starch and sugar fraction, (b) the alcohol-insoluble fraction excluding starch, and (c) the acid fraction, in all probability contribute to the loss of carbon in respiration.
2. The tension of both oxygen and of carbon dioxide affects the drain upon each of these fractions.
3. Oxygen tension has little effect upon the rate of cane sugar hydrolysis over the range studied.
4. Carbon dioxide increases the rate of cane sugar hydrolysis but decreases the rate of hydrolysis of the alcohol-insoluble fraction.
5. The results point to the existence of a fructosan in the alcohol-soluble fraction.
6. A comparative study of the starch + sugar fraction indicates that the behaviour of this fraction is anomalous, and that sugar must be both passing into and produced from the undetermined fraction during storage of the fruit.
7. There is a sharp contrast between the behaviour at 1 and at 5° C. as regards the alcohol-soluble undetermined fraction.
8. There is a production of fructose units during the hydrolysis of starch which is connected with this process.

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APPENDIX

Methods of analysis

The sample was extracted with hot 80% alcohol, and two fractions obtained:

- A. The alcohol-soluble portion was used for the determination of sugars and acids.
- B. The alcohol-insoluble portion was used for the determination of starch and residue.

A. *Acid.*

The syrup obtained by evaporation under reduced pressure of the alcoholic extract was made up to volume in water, and a suitable portion titrated with $N/10$ NaOH, using phenolphthalein as indicator. Acidity was calculated as malic acid.

Sugars. The water solution used for acid determination was neutralized and cleared with basic lead acetate and de-leaded with sodium phosphate. Sugars were estimated in this cleared solution using the Micro Shaffer-Hartmann method as modified by Dr Maskell:

Total sugar (hydrolysis with HCL) – reducing sugar = *sucrose*.

Fructose was estimated after oxidation of glucose with alkaline iodine solution:

Reducing sugar – fructose = *glucose*.

B. *Residue.*

Alcohol-insoluble residue was weighed after drying at 100° C. *Starch* was estimated on this dried residue using taka-diastrase as hydrolysing enzyme.

Primary chemical data for the flesh (cores and skins removed)

Days	Relative fresh weight	Glucose	Fructose	Sucrose	Starch	Acid	Residue including starch	% of brown or yellow-brown tissue
Initials								
0	100	1.001	5.556	2.837	0.610	1.052	2.281	0
0	100	1.229	5.762	2.606	0.618	1.125	2.357	0
0	100	1.175	5.539	2.702	0.668	1.095	2.379	0
0	100	1.087	5.709	2.847	0.680	1.110	2.355	0
0	100	0.956	5.740	2.814	0.655	1.172	2.424	0
0	100	0.944	5.554	2.890	0.652	1.150	2.359	0
0	100	1.004	5.728	2.865	0.597	1.107	2.338	0
0	100	1.111	5.622	2.813	0.597	1.083	2.303	0
0	100	1.201	5.550	2.734	0.642	1.091	2.320	0
0	100	1.038	5.706	2.877	0.672	1.091	2.417	0
Mean		1.075	5.647	2.798	0.639	1.108	2.353	0
Stored at 1° C., 0% CO ₂ , 2.5% O ₂								
26	99.92	1.127	5.972	2.707	0.171	1.072	1.879	0
69	99.67	1.413	6.177	2.325	0.119	1.002	1.744	0
113	99.55	1.415	6.032	2.247	—	0.987	1.671	1
138	99.45	1.702	5.800	1.889	—	0.944	1.713	1½
173	99.27	1.940	6.084	1.547	0.120	0.874	1.676	0
219	99.03	1.961	6.286	1.311	0.143	0.757	1.630	10½
Stored at 1° C., 0% CO ₂ , 5% O ₂								
26	99.93	1.442	5.778	2.646	0.184	1.088	1.900	0
69	99.67	1.549	5.917	2.311	0.119	1.010	1.737	0
113	99.62	1.582	6.116	2.196	—	0.996	1.761	1
138	99.46	1.710	6.120	1.728	—	0.947	1.709	½
173	99.29	1.664	6.372	1.563	0.118	0.877	1.667	0
219	99.03	1.855	6.101	1.507	0.143	0.786	1.660	15
Stored at 1° C., 0% CO ₂ , 10% O ₂								
26	99.92	1.189	5.814	2.574	0.149	1.062	1.812	0
69	99.67	1.363	5.902	2.383	0.118	1.036	1.733	0
113	99.63	1.605	6.008	2.331	—	0.979	1.702	0
138	99.50	1.765	6.055	1.882	—	0.952	1.686	0
173	99.33	1.797	6.252	1.397	0.119	0.834	1.634	0
219	99.20	1.721	6.369	1.071	0.131	0.753	1.666	4
Stored at 1° C., 0% CO ₂ , 21% O ₂								
26	99.60	1.292	5.663	2.732	0.153	1.082	1.785	0
69	98.85	1.546	5.756	2.452	0.118	1.054	1.749	0
113	98.60	1.446	5.953	2.078	—	0.920	1.694	0
138	98.14	2.038	5.777	2.393	—	0.875	1.657	0
173	97.81	1.860	6.132	1.433	0.122	0.777	1.614	0
219	97.36	1.861	6.191	1.178	0.117	0.693	1.591	2
Stored at 1° C., 5% CO ₂ , 10% O ₂								
26	99.58	1.187	5.755	2.867	0.158	1.064	1.828	0
69	98.90	1.505	6.108	2.311	0.112	1.031	1.785	0
113	98.63	1.784	5.999	2.097	—	0.931	1.730	0
138	98.36	1.513	6.295	1.787	—	0.896	1.679	3½
173	98.06	1.807	6.399	1.647	0.118	0.821	1.675	3½
219	97.62	1.830	6.551	1.275	0.127	0.781	1.707	20
Stored at 1° C., 10% CO ₂ , 10% O ₂								
26	99.61	1.222	5.660	2.670	0.146	1.112	1.862	0
69	98.87	1.474	6.133	2.312	0.121	1.019	1.829	0
113	98.60	2.035	5.659	1.582	—	0.908	1.692	1½
138	98.27	1.496	6.244	1.805	—	0.840	1.714	20
173	97.96	1.626	6.306	1.818	0.126	0.811	1.729	17½
219	97.44	1.883	6.225	1.982	0.113	0.744	1.755	43

Primary chemical data for the flesh (cores and skins removed) (contd.)

Days	Relative fresh weight	Glucose	Fructose	Sucrose	Starch	Acid	Residue including starch	% of brown or yellow- brown tissue
Stored at 1° C., 15 % CO ₂ , 10 % O ₂								
26	99.63	1.377	5.694	2.511	0.134	1.052	1.829	0
69	98.85	1.529	5.954	2.068	0.118	0.908	1.752	0
113	98.50	1.662	6.133	2.118	—	0.864	1.775	2½
138	98.13	1.711	6.229	1.811	—	0.809	1.778	7
173	97.63	1.600	6.185	1.812	0.123	0.744	1.827	33
219	97.04	1.703	5.890	1.648	0.102	0.536	1.806	54
Stored at 5° C., 0 % CO ₂ , 25 % O ₂								
26	99.48	1.640	6.184	2.584	0.144	1.087	1.820	0
69	98.76	2.344	5.827	1.318	0.127	0.938	1.672	0
113	98.47	1.946	6.555	1.280	—	0.855	1.626	0
138	97.88	2.060	6.715	1.275	—	0.881	1.672	0
173	97.56	2.144	6.879	0.883	0.122	0.840	1.663	0
219	97.13	1.990	6.888	0.677	0.134	0.759	1.612	0
Stored at 5° C., 0 % CO ₂ , 5 % O ₂								
26	99.43	1.261	6.173	2.671	0.118	1.023	1.774	0
69	98.73	1.536	6.060	2.054	0.124	1.023	1.710	0
113	98.23	1.850	6.316	1.408	—	0.894	1.615	0
138	97.76	1.856	6.651	1.214	—	0.836	1.627	0
173	97.40	1.745	6.564	0.874	0.122	0.815	1.629	0
219	96.84	2.028	6.598	0.686	0.134	0.684	1.578	0
Stored at 5° C., 0 % CO ₂ , 10 % O ₂								
26	99.42	1.650	5.951	2.387	0.119	1.027	1.734	0
69	98.39	1.547	6.188	2.080	—	0.968	1.698	0
113	97.88	1.888	6.321	1.601	—	0.870	1.633	0
138	97.43	1.947	6.296	1.257	—	0.806	1.620	0
173	96.88	1.718	6.524	0.821	0.119	0.781	1.644	0
219	96.01	1.823	6.292	0.756	0.116	0.638	1.546	4
Stored at 5° C., 0 % CO ₂ , 21 % O ₂								
26	99.35	1.358	5.845	2.519	0.111	1.036	1.731	0
69	98.60	1.410	6.153	2.238	—	0.949	1.710	0
113	98.29	1.596	5.943	1.517	—	0.835	1.604	0
138	97.73	1.833	6.087	1.387	—	0.803	1.642	0
173	97.42	1.900	6.034	1.022	0.125	0.702	1.594	3
219	96.76	1.818	6.038	0.652	0.126	0.618	1.562	8½
Stored at 5° C., 5 % CO ₂ , 10 % O ₂								
26	99.39	1.217	6.038	2.800	0.122	1.100	1.795	0
69	98.69	1.681	6.313	1.771	—	0.915	1.723	0
113	98.32	1.767	6.425	1.433	—	0.887	1.685	0
138	98.20	1.857	6.347	1.286	—	0.869	1.672	0
173	98.14	2.027	6.957	1.006	0.119	0.812	1.732	0
219	97.35	2.050	6.918	0.528	0.116	0.733	1.692	0
Stored at 5° C., 10 % CO ₂ , 10 % O ₂								
26	99.38	1.437	6.224	2.463	0.132	1.002	1.816	0
69	98.76	2.065	6.004	1.731	0.106	0.927	1.765	0
113	98.48	1.733	6.528	1.419	—	0.910	1.740	0
138	98.05	1.909	6.553	1.182	—	0.835	1.686	0
173	97.87	1.746	7.096	1.117	0.112	0.851	1.760	0
219	97.59	2.203	6.571	0.751	0.118	0.749	1.652	0
Stored at 5° C., 15 % CO ₂ , 10 % O ₂								
26	99.41	1.470	6.040	2.471	0.144	1.022	1.786	0
69	98.75	1.680	6.235	1.826	0.119	0.962	1.737	0
113	98.38	1.978	6.238	1.185	—	0.869	1.683	0
138	97.99	1.710	7.050	0.988	—	0.806	1.686	0
173	97.74	1.536	6.958	0.878	0.124	0.795	1.693	0
219	97.37	2.019	6.780	0.503	0.103	0.718	1.670	0

FORMATION OF "FRUCTOSE" UNITS DURING STARCH HYDROLYSIS IN THE LATER STAGES OF THE GROWTH OF THE APPLE

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RESULTS calculated from a critical set of data by Archbold (1932) on the chemical composition of Bramley's Seedling apples during their development and from our own observations on the rate of CO_2 -production by apples of the same variety at different stages of growth are given in the table below.

Four stages in the ontogeny of the apple are clearly recognizable:

- (a) Cell division stage which ends about the middle of June.
- (b) Cell enlargement, first phase, up to point of maximum starch accumulation.
- (c) Cell enlargement, second phase, during loss of starch up to the climacteric.
- (d) Senescent or post-climacteric stage.

For the first three of these stages we find the following results (grams per average apple) if we class protein and cell-wall material with the "glucose" fraction and the undetermined with the "fructose" fraction.

Increments	"Glucose" fraction	"Fructose" fraction	Acid	Respiratory loss of carbon expressed as hexose
Stage a: 1-22 days (27. v. 30-17. vi. 30)	0.303	0.285	0.090	0.090
Stage b: 22-77 days (17. vi. 30-11. viii. 30)	4.136	4.494	1.136	0.900
Stage c: 77-148 days (11. viii. 30-21. x. 30)	2.546	7.004	0.526	2.010

The work of Mason & Maskell (1928) on the cotton plant provides good grounds for assuming, in the absence of any direct evidence in the case of the apple, that the carbohydrate of transport to the fruit is sucrose. Making this assumption, the above values indicate that there is no extensive interconversion of "glucose" units to "fructose" units or the reverse except when starch hydrolysis is known to be proceeding. There is then an active conversion of "glucose" to "fructose".

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Data in grams per mean apple for the growth of Bramley's Seedling apples

Days from 27 May 1930	Dry weight	Fructose	Glucose	Sucrose	Starch	Cell wall*	Acid	"Protein"	Un- identified fraction†	Surface	Respiratory loss as sugar for average temperature of 15° C.	Mean wt. per apple g.
22	0.59	0.033	0.040	0.018	0.007	0.19	0.091	0.064	0.167	15.6	0.09	5.77
29	1.31	0.102	0.109	0.063	0.016	0.38	0.255	0.106	0.340	27.1	—	13.31
38	2.57	0.309	0.234	0.104	0.094	0.60	0.498	0.162	0.520	41.3	—	24.88
49	4.04	0.583	0.422	0.265	0.316	0.91	0.696	0.200	0.620	54.3	—	37.64
63	5.99	1.170	0.653	0.745	0.561	1.21	0.937	0.243	0.690	68.5	—	54.35
77	9.22	2.170	0.943	1.208	0.990	1.62	1.228	0.289	0.720	88.6	0.99	78.50
92	10.65	2.950	1.020	1.695	1.285	1.63	1.250	0.308	0.790	95.0	—	87.94
105	12.40	3.820	1.244	2.130	0.930	1.95	1.300	0.320	0.910	105.0	—	101.89
119	15.20	4.810	1.440	3.130	0.860	2.12	1.410	0.350	1.080	120.0	—	123.10
134	17.37	5.670	2.100	4.050	0.180	2.29	1.610	0.388	1.280	128.6	—	138.17
148	18.77	6.590	2.010	4.380	0.000	2.30	1.740	0.422	1.460	150.5	3.00	150.68

* This fraction is obtained from the difference between dry weight and sum of determined components (i.e. cane sugar, fructose, glucose, acid, starch, protein and cell-wall material).

† These values are obtained from the values for alcohol-insoluble residue by subtraction of the appropriate value for insoluble protein. Data obtained by Hulme (1934) show that the insoluble nitrogen approximates to 60% of the total throughout the period of cell enlargement.

STUDIES ON THE SULPHUR METABOLISM OF PLANTS

I. PRELIMINARY INVESTIGATIONS ON THE EFFECTS OF DIFFERENT EXTERNAL CONCENTRATIONS OF SUL- PHATE, AMMONIA AND CYSTINE ON THE AMOUNTS OF SULPHUR-CONTAINING COMPOUNDS IN LEAVES

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(With 5 figures in the text)

INTRODUCTION

IN this paper are presented the results of a preliminary exploration of the sequence of reactions involved in the sulphur metabolism of plants, and of the relations which exist between the amounts of the metabolites.

In order to achieve this end we have employed the experimental method developed by Petrie & Wood (1938a); in its essentials this method entails alteration of the internal concentration of metabolites by applying to plants different treatments of one of them and then placing the treated plants in an environment constant in certain respects. Under these conditions it is possible that the systems in the plant will approximate to a drifting steady state. At this state it might be expected that the factors determining the amounts present of substances taking part in the metabolic reactions would be more clearly revealed. At intervals a certain number of plants corresponding to each treatment were removed and the amounts of various components of the system estimated by appropriate analytical methods.

In this paper we are concerned only with the system present in the leaves and describe the effects of adding different dosages of potassium sulphate, ammonium chloride and cystine to the medium in which plants were growing.

THE REACTIONS INVOLVED

References to the sulphur metabolism of plants are few, and examination of these reveals that not only is there lack of agreement concerning the chemical units which may enter the metabolic sequences but also uncertainty as to the sequence of reactions involved.

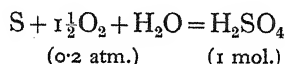
A survey of the literature dealing with the sulphur metabolism of plants as well as analogies with the sulphur metabolism of animals, suggests that the following sulphur compounds may be metabolites: inorganic sulphates; "conjugated" or ethereal sulphates, such as phenosulphonic acids; sulphur-containing proteins; the

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two sulphur-containing amino-acids, cystine and methionine, which are known constituents of the protein molecule; and probable derivatives of protein or of the sulphur-containing amino-acids, such as glutathione and cysteine.

It is clear that inorganic sulphates constitute the only source of sulphur to plants grown under normal conditions, and that sulphur-containing proteins are the synthetic end-products. The sequence of events resulting in protein formation is not clearly defined and two alternative hypotheses have been proposed to account for protein synthesis. According to the first view, amino-acids are precursors of proteins, and are formed by combination of ammonia with carbohydrate residues produced during glycolysis of sugars, and in the case of cystine with a sulphide residue as well. The second hypothesis states that proteins are formed *en bloc* from the above-mentioned complexes and that the amino-acids are derived solely from hydrolysis of proteins. From the evidence available at the present time it is impossible to distinguish between the conflicting theories.

Whatever view is accepted it is apparent that reduction of sulphate to sulphide must occur. The reaction:



can occur spontaneously; the free-energy change is negative, and under the above conditions has the value $\Delta F_{298} = -118,500$ cal. Among living organisms this reaction occurs during metabolism of the *Thioxidans* group of sulphur bacteria. The reverse reaction, the reduction of sulphate to sulphide, is also brought about by anaerobic sulphate-reducing bacteria. Here the reduction proceeds with an increase in free energy which, it is generally assumed, is supplied during the oxidation of carbohydrate residues in respiration; furthermore, in the case of the bacteria the reduction proceeds only in alkaline media at about pH 8.

The source of energy for sulphate reduction in green plants is obscure. Among alternatives which might provide a source of energy are included: oxidation of carbohydrate residues in respiration; the radiant energy in a photochemical reduction; combination of sulphate ions with a large molecule or micelle which by intramolecular rearrangement might supply energy for reduction. At present the experimental evidence is not sufficiently conclusive to permit differentiation between these alternatives. Certain authors (Eaton, 1935; Heiserich, 1935) have attempted to correlate the amount of reduction of sulphate with the amount of a "reductase" present, but there is no experimental evidence that such an enzymatic reduction of sulphate occurs. In formulating a scheme suggesting the most likely sequences concerned in sulphur metabolism we have simply assumed that sulphate is reduced to sulphide.

In the animal organism there is abundant evidence that sulphate is formed by oxidation of organic sulphur compounds. If a high-protein diet is fed to animals, the sulphur is eliminated in oxidized form as sulphate; the bulk as inorganic sulphate and a small fraction in ester linkage as "conjugated" or ethereal sulphuric acids which are precipitated by barium chloride only after hydrolysis with acids. In this oxidation process substances such as chondroitin sulphate or taurocholic acid may

be intermediate compounds, but these compounds have not been detected in plant organisms.

In the animal organism cystine is also oxidized to sulphate, and the same result may be obtained *in vitro* by the action of nitrous acid or hydrogen peroxide on cystine. Cystine may also be oxidized to the sulphonic acid cysteic acid, but the resistance to further oxidation of this substance, both *in vivo* and *in vitro*, makes it unlikely that biological oxidations follow this course. White (1933) has prepared peptides of sulphonic acids which also are not oxidized *in vivo*.

Experimental data sufficient to explain the mechanism of oxidation of cystine are lacking. Experiments *in vivo* and *in vitro* do not run parallel; *in vitro* it is possible to oxidize cystine without affecting the amino group, but if the amino group is "blocked", as, for example, by combination with a phenylureido or benzoyl group, no oxidation of sulphur occurs in the living organism. Similar experiments with S-substituted cysteines, such as S-benzoyl cysteine or S-ethyl cysteine, show that a second essential for oxidation is a free sulphydryl group (Lewis, 1935). The most effective point of attack for oxidation of cystine is the amino group, and the second the sulphydryl group. It would appear from these data that free cystine only is oxidized *in vivo* and not protein sulphur or methionine.

Mothes & Specht (1934) have claimed that in plants cystine is oxidized to an ethereal sulphate which they regard as forming the "sulphur reserve" of the plant. It should be stated here that in the large number of determinations which have been made in the course of our experiments we have not been able to detect any ethereal sulphates in leaves; the whole of the sulphate present exists in inorganic form.

Cysteine is formed from cystine by reduction and is also readily oxidized to cystine. Its occurrence as a primary unit of protein structure is problematical. The analytical estimation of cysteine in mixtures containing cystine presents difficulties which have not yet been overcome, but under the conditions of experimental technique used by us only cystine is present in the plant extracts.

The oxidized and reduced forms of the sulphur-containing tripeptide glutathione also occur in plant tissues. In the experiments described here we have been unable to detect glutathione in the plant extracts used.

From a review of the above-mentioned facts we present the following scheme as a working hypothesis to explain the plexus of reactions making up the sulphur metabolism of the plant:

- (1) The arrival of the ammonia nitrogen and sulphate sulphur in the leaves.
- (2) The formation of glycolysis products in respiration and their disappearance in oxidation to carbon dioxide, to organic acids and in other ways.
- (3) The reduction of sulphate ions to sulphide and the reoxidation of sulphide to sulphate.
- (4) The combination of compounds, derived from glycolysis with ammonia nitrogen to form amino-acids and amides, and their deamination and deamidation to reform ammonia nitrogen and carbon residues.
- (5) Included in (4) is the combination of ammonia nitrogen and residues from glycolytic products with the sulphide formed by sulphate reduction to form cystine

and the oxidation of cystine to reform sulphide, ammonia nitrogen and organic acids or other carbon residues.

(6) The synthesis, in a number of stages, of proteins from the amino-acids so formed. As mentioned above there is no evidence which enables us to distinguish between this method of formation of proteins and that of *en bloc* condensation.

(7) The translocation of synthesized compounds away from the leaf.

This scheme is simply an extension of that put forward by Petrie & Wood (1938*a*) for the sequence of reactions in nitrogen metabolism, by the inclusion of the reactions concerned in the transformation of the sulphur compounds. The scheme implies a network of balanced reactions, some truly reversible, others opposed with regard to their beginning and end-points, but possibly proceeding along different paths. In a system such as this, a change in concentration of one component will affect the concentration of the other components of the system; but it is possible that some substances may never reach equilibrium but are maintained at non-equilibrium concentrations by the expenditure of energy by the living system.

DESCRIPTION OF EXPERIMENTS

(1) *Analytical technique*

The plants after sojourn under constant conditions were prepared for analysis in the manner described by Petrie & Wood (1938*a*). The technique developed for estimation of nitrogen fractions has also been described in that paper. The methods used for estimation of sulphur fractions are described below.

The common protein precipitants, tungstic acid, phosphotungstic acid and uranyl salts cannot be used in the estimation of protein sulphur, nor can the filtrate obtained after removing protein with these precipitants be used for the estimation of soluble sulphur compounds, since the subsequent addition of barium chloride precipitates insoluble barium compounds.

Trichloroacetic acid in 10 or 20% solution, widely used as a protein precipitant in blood analysis, has been tried; this proved quite unsatisfactory for the plant proteins of the grasses used and brought about flocculation only on prolonged standing. Also the filtrate from the trichloroacetic acid precipitates could not be used for the estimation of cystine. In a series of six different analyses no cystine could be detected in trichloroacetic acid extracts, although when tungstic acid was used as a precipitant appreciable amounts of cystine were obtained from the same materials. This is probably caused by traces of mono- and dichloroacetic acids in the trichloroacetic acid, since Dickens (1933) has shown that the two former acids react with cysteine and also with glutathione, the reaction freeing halogen acids and introducing a sulphydryl group into the acetic acid.

Ultimately sulphur-free tannin, dissolved in 1% hydrochloric acid to give a 4% solution, was used as a protein precipitant. This reagent completely precipitated the protein and gave closely agreeing results for protein sulphur and also for soluble sulphur fractions in replicated analyses. Cystine could not be estimated in the extract owing to the dark colour of the oxidized tannin solution and also because

tannin reduces the Folin-Marenzi reagent. Cystine sulphur, therefore, was always estimated in the filtrate from a duplicate sample in which protein was precipitated with tungstic acid.

The procedure adopted was to grind weighed samples of the fresh leaf material to a smooth paste with pure quartz sand and 40 ml. of 4% tannin solution. As little as possible of the tannin solution was used in the actual grinding process. After the grinding had been completed the rest of the tannin solution was warmed to 40° C. and added to the ground material which was then stirred and allowed to stand for 10 min. before filtering. The paste was then filtered through no. 44 Whatman filter papers in a Buchner funnel and soluble sulphur fractions estimated in aliquots of the filtrate. After collection of the filtrate the precipitate was well washed with 1% tannin solution and protein sulphur determined in this precipitate.

Protein sulphur. For determination of protein sulphur the wet digestion method of Benedict-Denis as modified by Frear (1930) was adopted after preliminary trials. This method, as well as other standard methods for the determination of organic sulphur, has recently been criticized. Painter & Franke (1936), in a comparative study of the results given by the Benedict-Denis method and oxidation in a Parr calorimeter bomb, found that the latter method gave higher values than the former with both cereal and protein samples. They also claimed that whilst practically all the cystine sulphur is oxidized by the Benedict-Denis method only about one-third of the methionine sulphur is oxidized. Marston (1937) has also emphasized the discrepancy between various methods for sulphur analysis when different materials are used. He decided that the only method which gave consistent results was oxidation with compressed oxygen in a bomb; he found, however, that although the Benedict-Denis method gave very low results with animal excreta it gave the same results as the bomb method with dried fodder. It proved difficult to use the bomb method with our protein precipitants since they contain about 5 g. of sand used in the grinding process.

Protein sulphur in dried leaf material was determined in an Emerson bomb and gave mean values for duplicate samples which agreed within 2% of those obtained by the Benedict-Denis method. But whereas duplicate analyses by the bomb method agree to within 1%, the duplicates using the Benedict-Denis method show a range of variation of about 7%.

A further difficulty is encountered when the sulphate sulphur content varies considerably, for some of the sulphate is adsorbed on the protein precipitate. In a test experiment varying amounts of potassium sulphates were added during the grinding process to replicated samples, each of 10 g. fresh weight of a grass; protein sulphur was determined according to the usual procedure with the following results:

Potassium sulphate added g.	Protein sulphur % dry weight
0	0.190
0.03	0.216
0.04	0.230

Inorganic sulphate sulphur. This was determined in 15–20 ml. of tannin filtrate by adding 10 ml. 10% barium chloride to the cold filtrate and allowing the mixture to stand over night. The sulphate was precipitated as soon as possible after preparation of the extract in order to avoid hydrolysis of any labile ethereal sulphates which might be present. In addition to barium sulphate, other compounds, especially barium salts of amino-acids, are precipitated, but the latter can be removed quantitatively by washing the precipitate with hot dilute hydrochloric acid. An attempt was made to overcome this difficulty by estimating sulphate sulphur colorimetrically after precipitating with benzidine, diazotizing and coupling with thymol according to the method of Cuthbertson & Tompsett (1931). The method proved unsatisfactory, since other substances present in the extract precipitate the benzidine and the presence of phenolic substances gave impure colours after diazotization.

After experience in earlier trials had proved that ethereal sulphates were not present in the leaves, the sulphate was precipitated by barium chloride in boiling dilute acid (0.02*N*) solution.

Ethereal sulphate sulphur. 15–20 ml. of the filtrate was used and 2.5 ml. 6*N* hydrochloric acid added for each 10 ml. of filtrate. The mixture was hydrolysed at 60° C. for 24 hr. and barium chloride then added to the boiling solution. The precipitate was then washed with boiling dilute acid as before. The ethereal sulphate was estimated by deducting the inorganic sulphate previously determined from the value obtained by this method.

Cystine sulphur. Records of determinations of the reduced sulphur compounds, cystine, cysteine and glutathione, in plant tissues are few, and some consideration of methods is necessary. The only previous attempt to determine reduced sulphur in plant extracts was by Heiserich (1935) who used Kühnau's method (1931), which is a modification of the iodine thiosulphate method first proposed by Dixon & Tunnicliffe (1923) for glutathione. This method is unreliable, since it is now known that other substances present in plant extracts, notably ascorbic acid, absorb iodine. The method therefore gives high results when expressed as reduced sulphur. The technique used by Heiserich is also faulty, since the reduction of the oxidized forms of glutathione or cystine follows the following equation, as was shown by Pulewka and Winzer (1928):



Only one-half of the total disulphide sulphur is estimated, and furthermore, the reaction only occurs at pH 8 or greater. The reaction is imperceptible in the acid solutions used by Heiserich. The method was modified by us to give approximate results as follows: Two aliquots of the filtrate were taken and 1 ml. of 0.1*M* mercuric sulphate solution, which precipitates cystine, cysteine and glutathione, added to one of them. The solutions were adjusted to pH 0.5, sodium cyanide and iodine were added and allowed to react in the dark for 1 hr. The difference between the titrations of the two aliquots with thiosulphate gives a measure of half the disulphide sulphur. However, the differences between the titrations in our material was so small that little reliance could be placed upon the value obtained.

Finally, Lugg's modification of the Folin-Marenzi method (1932) and Lugg's modification of Sullivan's method (1933) were adopted with satisfactory results. In these modified methods the pH is strictly controlled throughout the course of the estimations. The former is a general method and estimates all sulphhydryl and disulphide groups present. Sullivan's method is specific for cysteine, but it is necessary to centrifuge before colorimetric comparison, since magnesium salts form a coloured lake with the naphthoquinone sulphonate used; compensation must also be allowed for the colour of tungstic acid extracts. The amounts of reduced sulphur in our extracts were so small as to be towards the limits of applicability of the method, but a micro-modification, entailing the use of a total volume of 10 ml. instead of 100 ml., enabled satisfactory estimations to be made.

In the extracts used by us it was not possible to detect any cysteine, only cystine being present. Presumably during the grinding process any cysteine present is oxidized to cystine, therefore care was taken to ensure that the time of grinding was the same in each case.

Extraneous reducers other than cystine produce a colour with the Folin-Marenzi reagent. These reducers affect the accuracy of estimation when the amount of cystine is small, since the method depends on the difference in depths of colour produced when cystine is (a) present, and (b) eliminated from the solution. In plant extracts much of the colour produced appears to be due to pyruvic acid and methyl glyoxal. The greater part of the extraneous reducing substances were removed by shaking aliquots of the extracts with ether which partially removes them but does not dissolve the cystine which was then determined in the aqueous solution.

Table I gives the results of analyses for free cystine by different methods; the agreement obtained by the use of the modified Folin-Marenzi and Sullivan methods is satisfactory.

Table I. *Comparison of amounts of cystine estimated by different methods (Exp. C.)*

Day	Treatment	Milligrams of cystine in 1 ml. of tungstic acid extract	
		Folin-Marenzi method	Sullivan method
1	I	0.0034	0.0034
	II	0.0041	0.0036
	III	0.0046	0.0041
	IV	0.0047	0.0041
1½	V	0.0037	0.0035
	VI	0.0054	0.0040
	VII	0.0064	0.0042
	VIII	0.0045	0.0040
2	I	0.0032	0.0037
	II	0.0058	0.0040
	III	0.0036	0.0042
	IV	0.0072	0.0042
	V	0.0036	0.0039
	VI	0.0025	0.0045
	VII	0.0054	0.0050
	VIII	0.0055	0.0046

Total soluble sulphur. This was determined in 15 ml. of the tannin filtrate using the Benedict-Denis method.

We have found in each separate treatment in our experiments that the total soluble sulphur is in excess of the sulphate sulphur plus cystine sulphur. This excess is probably due to methionine which, according to Pollard & Chibnall (1934), is present in fairly large amounts in grass proteins. At present methionine cannot be estimated satisfactorily in complex solutions containing other methoxy compounds. Gravimetric methods in which methionine is estimated by difference are unreliable where the amount present is as small as that indicated in our solutions; consequently we have not taken this amino-acid into consideration in our experiments.

Total sulphur was estimated as the sum of the protein sulphur and total soluble sulphur.

All the data are expressed as percentage of the dry weight, and in each case are the means of analyses of duplicate samples of leaf material.

(2) *Experimental method*

The experimental procedure was similar to that described by Petrie & Wood (1938a) for experiments on the nitrogen metabolism of grasses. Seeds of a pure line of grass were sown in glass jars containing 3.75 kg. water-washed sand maintained with distilled water at 70% of its saturation capacity. The culture solutions used were the same as in the experiments on nitrogen metabolism referred to above. In no case was the external sulphate supply low enough to bring about symptoms of sulphur deficiency in any of the plants; all nutrients were supplied in amount sufficient to carry the plants to maturity. In a preliminary experiment an attempt was made to grow large numbers of sulphur-deficient plants. The plants grew so unevenly and the mean dry weight of plants per pot varied so greatly that they could not be used for the type of experiment contemplated here and they were therefore discarded.

Experiment A. Seeds of *Phalaris tuberosa* L. and *Lolium multiflorum* Lam. were sown on 5 May 1935. Sojourn in the cabinets commenced on 3 September 1935 and the following treatments added in solution:

<i>Phalaris tuberosa</i> :	I	0 g.	K_2SO_4	per pot
	II	2.00 g.	" "	" "
<i>Lolium multiflorum</i> :	I	0 g.	K_2SO_4	per pot
	II	2.00 g.	" "	" "
	III	4.00 g.	" "	" "

Pots were removed for analysis on 5 and 6 September 1935. Four pots per treatment were used for analysis. The results of analysis are given in Table II.

Experiments B and C. Seeds of *Phalaris tuberosa* L. were sown on 12 May 1936. Sojourn in the cabinets commenced on 30 August 1936. The constant environment in the cabinets was characterized as follows: light intensity, 432 m.c.; temperature, 24° C.; relative humidity, approximately 85%; the water content of the sand in the pots was maintained at 70% of its saturation capacity.

Table II. Result of Exp. A

All fractions are expressed as percentage of dry weight

Plant	Treatment	Water	Inorganic sulphate sulphur	Cystine sulphur	Protein sulphur	Total sulphur	Ammonia nitrogen	Residual Amino nitrogen	Protein nitrogen
<i>Phalaris tuberosa</i>	I	628	0.522	0.010	0.386	0.932	0.016	0.158	2.51
	II	628	0.647	0.010	0.387	1.029	0.018	0.162	2.57
<i>Lolium multiflorum</i>	Day 1								
	I	433	0.247	0.015	0.365	0.740	0.014	0.436	2.68
	II	430	0.285	0.015	0.358	0.775	0.014	0.445	2.57
	III	430	0.373	0.015	0.368	0.874	0.014	0.460	2.66
	Day 2								
	I	454	0.182	0.015	0.367	0.640	0.027	0.426	2.65
	II	445	0.291	0.017	0.378	0.767	0.022	0.404	2.59

Differential treatments were applied 24 hr. after the pots had been placed in the cabinets; pots were removed for analysis at intervals on the days following. Details of differential treatments and times of analysis in the individual experiments are set out below.

Experiment B. The following treatments were applied in solution 24 hr. after the pots had been placed in the cabinets:

I	0 g.	K_2SO_4	per pot
II	0.85 g.	"	"
III	2.55 g.	"	"
IV	4.45 g.	"	"

Owing to limitations of space all pots could not be placed in the cabinets at the same time. Pots designated "day 1" were placed in the cabinets on 30 August 1936, received differential treatments on 31 August, and were analysed on 1 September. Pots designated "day 2a" were placed in the cabinets on 1 September, received differential treatments on the same night and were analysed on 3 September; pots designated "day 2b" were placed in the cabinets on 2 September, received differential treatments the same night and were analysed on 4 September. On day 2b mechanical breakdown occurred of a fan which formed part of the circulatory system in the cabinets. This caused the relative humidity in the cabinet to rise. Four pots per treatment were used for analysis. The results are given in Tables III and V and Figs. 1 and 2.

Table III. Mean dry weights, Exp. B

Day	Treatment				Day mean
	I	II	III	IV	
1	3.43	3.50	3.30	3.43	3.42
2	3.27	3.26	3.38	3.32	3.31
3	3.50	3.45	3.40	3.42	3.44
Treatment means	3.40	3.40	3.37	3.39	3.39

Time, treatment and interaction insignificant.
Standard error of mean of four pots ± 0.080 .
Coefficient of variation 2.36.

Table IV. *Mean dry weights, Exp. C*

Day	Treatment								Day means
	I	II	III	IV	V	VI	VII	VIII	
I	3.43	3.42	3.42	3.34	3.36	3.37	3.20	3.14	3.34
2	3.56	3.32	3.59	3.49	3.39	3.48	3.25	3.56	3.45
Treatment means	3.50	3.37	3.50	3.42	3.38	3.43	3.23	3.35	3.40

Time, treatment and interaction insignificant.
 Standard error of mean of four pots ± 0.129 .
 Coefficient of variation 3.79.

Table V. *Results of Exp. B*

All fractions are expressed as percentages of dry weight

Treatment	Water	Inorganic sulphate sulphur	Cystine nitrogen	Protein sulphur	Total sulphur	Total amino nitrogen	Protein nitrogen	Protein nitrogen / Protein sulphur
Day 1 I	467	0.226	0.0013	0.178	0.421	0.071	2.30	12.9
II	463	0.306	0.0013	0.189	0.485	0.065	2.36	12.5
III	472	0.347	0.0015	0.204	0.546	0.069	2.32	11.9
IV	486	0.345	0.0016	0.185	0.560	0.074	2.31	12.5
Day 2 a I	502	0.214	0.0017	0.174	0.406	0.073	2.36	13.6
II	531	0.301	0.0020	0.194	0.495	0.081	2.50	12.9
III	500	0.400	0.0014	0.204	0.604	0.088	2.34	11.7
IV	494	0.410	0.0015	0.189	0.604	0.080	2.37	12.5
Day 2 b I	533	0.270	0.0019	0.198	0.477	0.093	2.48	12.5
II	533	0.306	0.0016	0.217	0.513	0.100	2.65	12.2
III	515	0.343	0.0015	0.211	0.554	0.091	2.51	12.1
IV	546	0.390	0.0020	0.213	0.603	0.100	2.58	12.1

Table VI. *Results of Exp. C*

All fractions are expressed as percentages of dry weight

Treatment	Water	In-organic sulphate sulphur	Total soluble sulphur	Organic soluble sulphur	Cystine nitrogen	Protein sulphur	Total sulphur	Total amino nitrogen	Protein nitrogen	Protein nitrogen / Protein sulphur
Day 1 I	467	0.226	0.243	0.017	0.001	0.178	0.421	0.071	2.30	13.0
II	483	0.239	0.279	0.040	0.001	0.201	0.480	0.076	2.47	12.4
III	477	0.273	0.248	0.025	0.001	0.189	0.437	0.067	2.39	12.6
IV	497	0.330	0.350	0.020	0.001	0.201	0.551	0.083	2.48	12.5
Day 1½ V	507	0.175	0.220	0.040	0.001	0.182	0.402	0.175	2.79	13.6
VI	490	0.192	0.272	0.080	0.002	0.187	0.402	0.180	2.68	13.7
VII	512	0.185	0.238	0.053	0.002	0.191	0.432	0.170	2.70	14.2
VIII	543	0.210	0.230	0.020	0.002	0.209	0.433	0.174	2.85	13.6
Day 2 I	487	0.221	0.256	0.035	0.001	0.162	0.418	0.059	2.28	14.0
II	498	0.224	0.250	0.026	0.001	0.179	0.429	0.070	2.45	13.7
III	457	0.225	0.245	0.020	0.001	0.166	0.411	0.073	2.24	13.5
IV	487	0.228	0.255	0.023	0.002	0.163	0.408	0.072	2.24	13.7
V	503	0.152	0.202	0.050	0.001	0.200	0.402	0.138	2.78	13.3
VI	498	0.163	0.208	0.041	0.002	0.194	0.402	0.134	2.65	14.3
VII	512	0.147	0.221	0.054	0.002	0.211	0.429	0.128	2.76	13.5
VIII	523	0.177	0.240	0.057	0.002	0.193	0.439	0.158	2.83	13.6

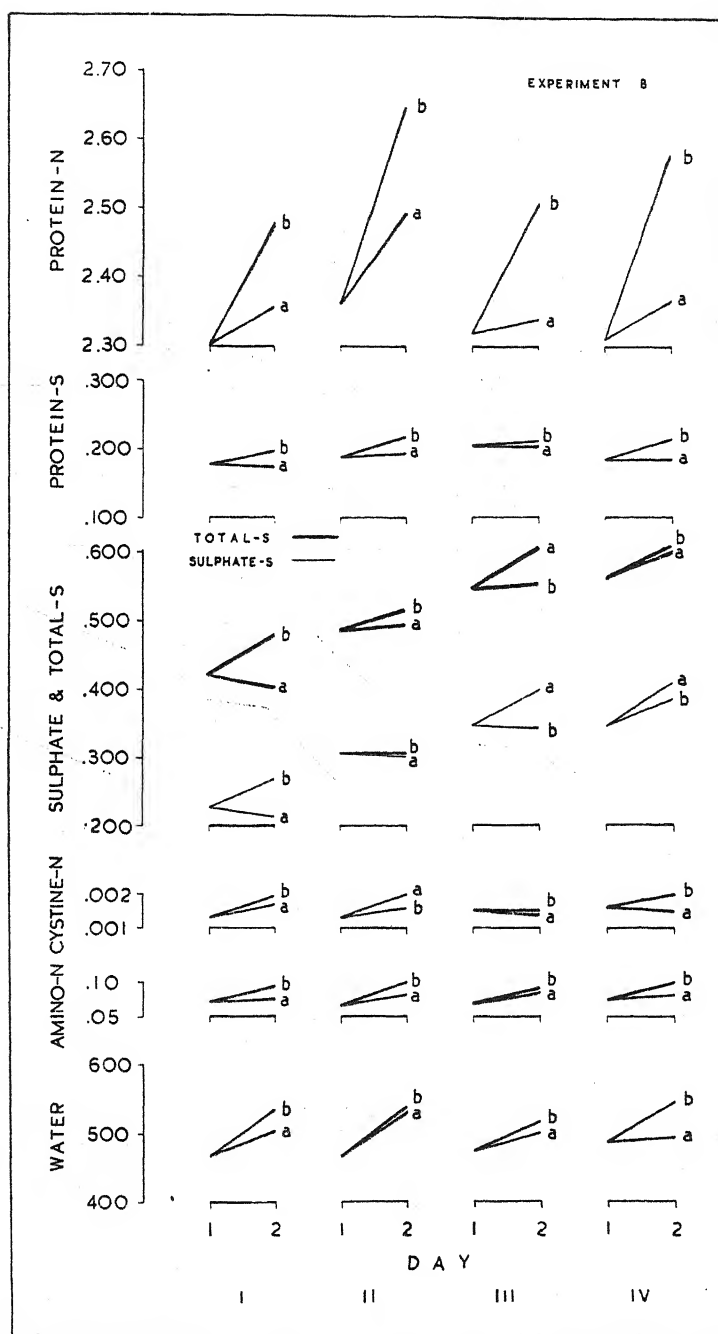


Fig. 1. Drifts with time in the contents of various components of the leaves, Exp. B. All quantities expressed as percentages of dry matter in this and subsequent figures.

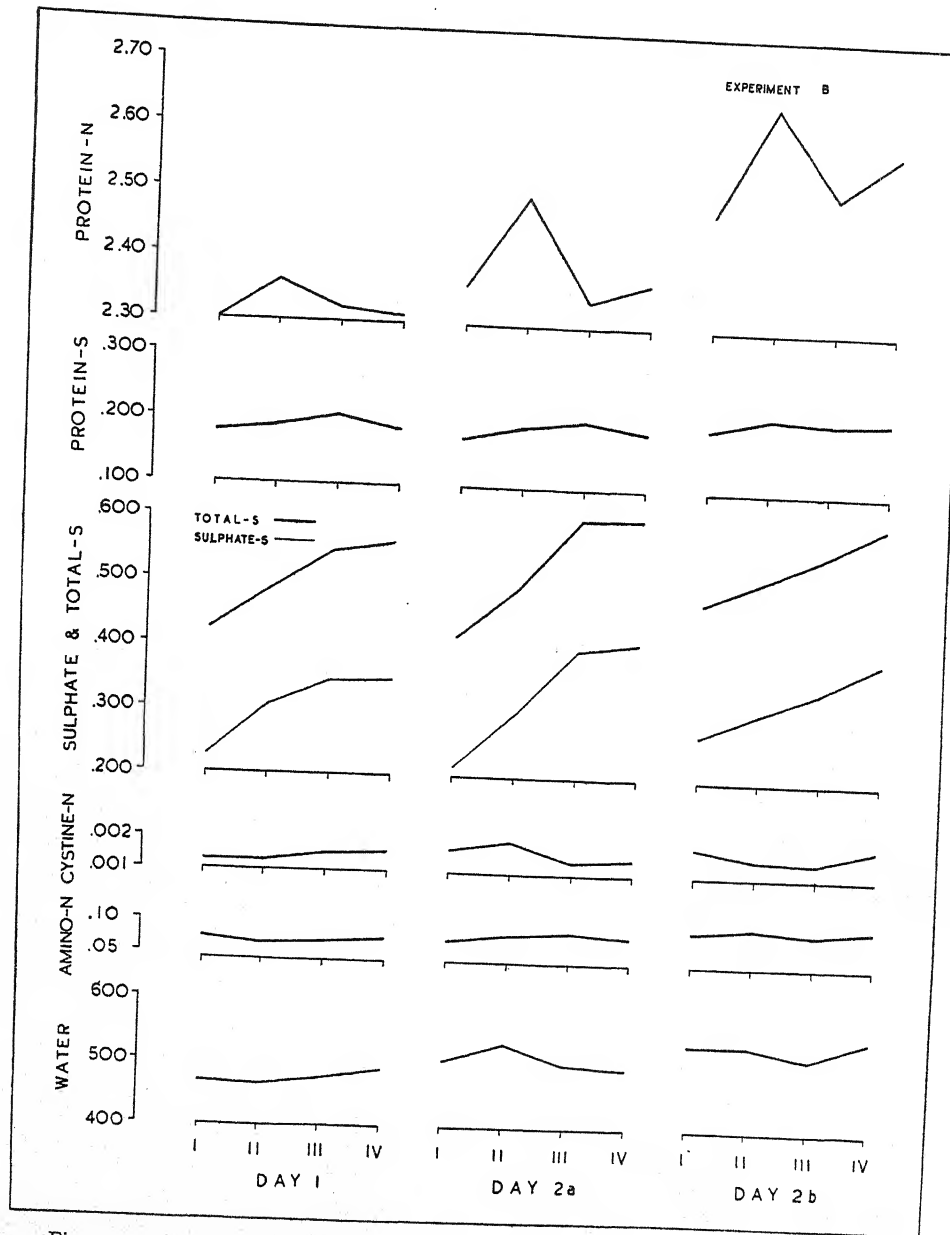


Fig. 2. Treatment effects on the contents of various components of the leaves, Exp. B.

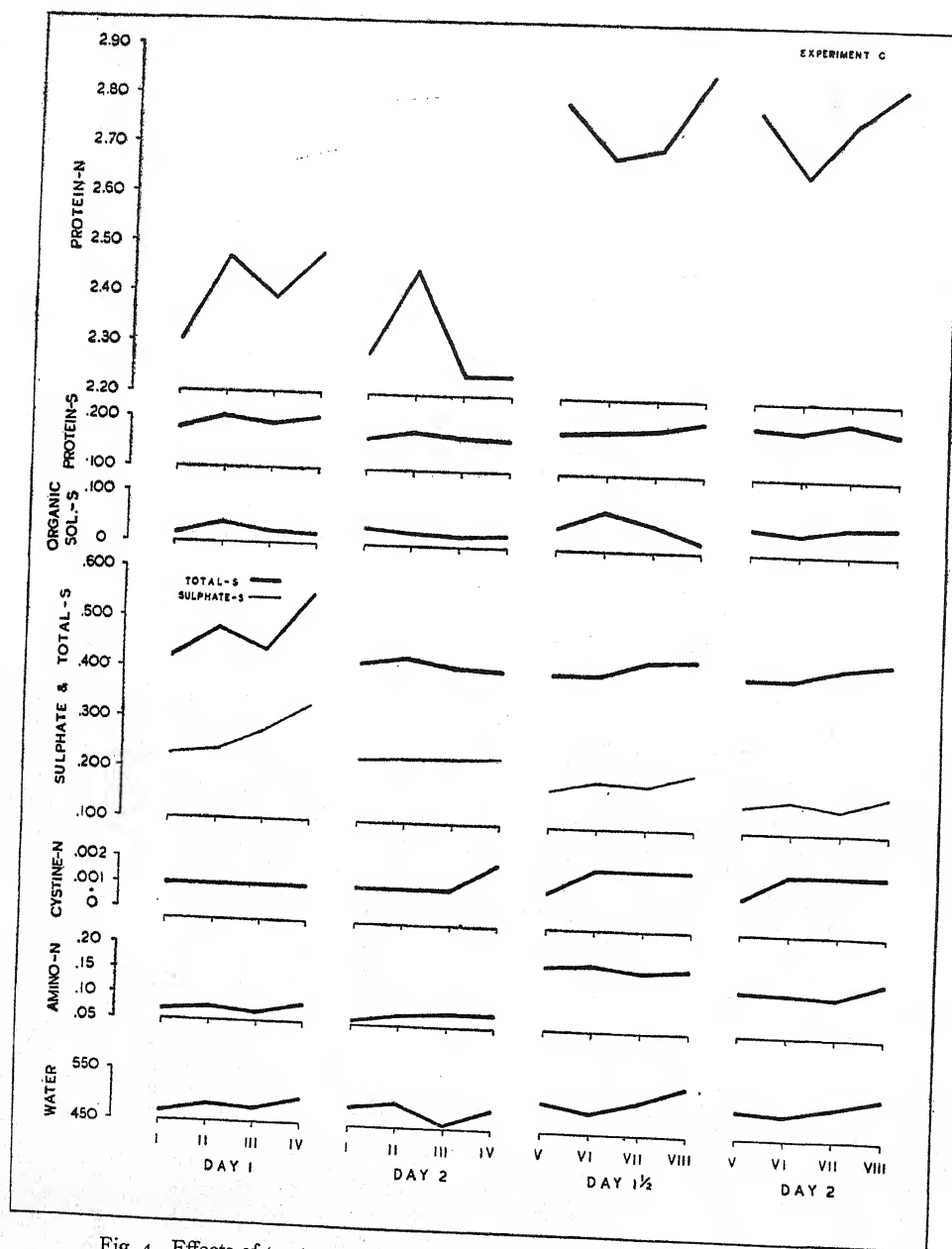


Fig. 4. Effects of treatment with cystine on the contents of various components of the leaves, Exp. C.

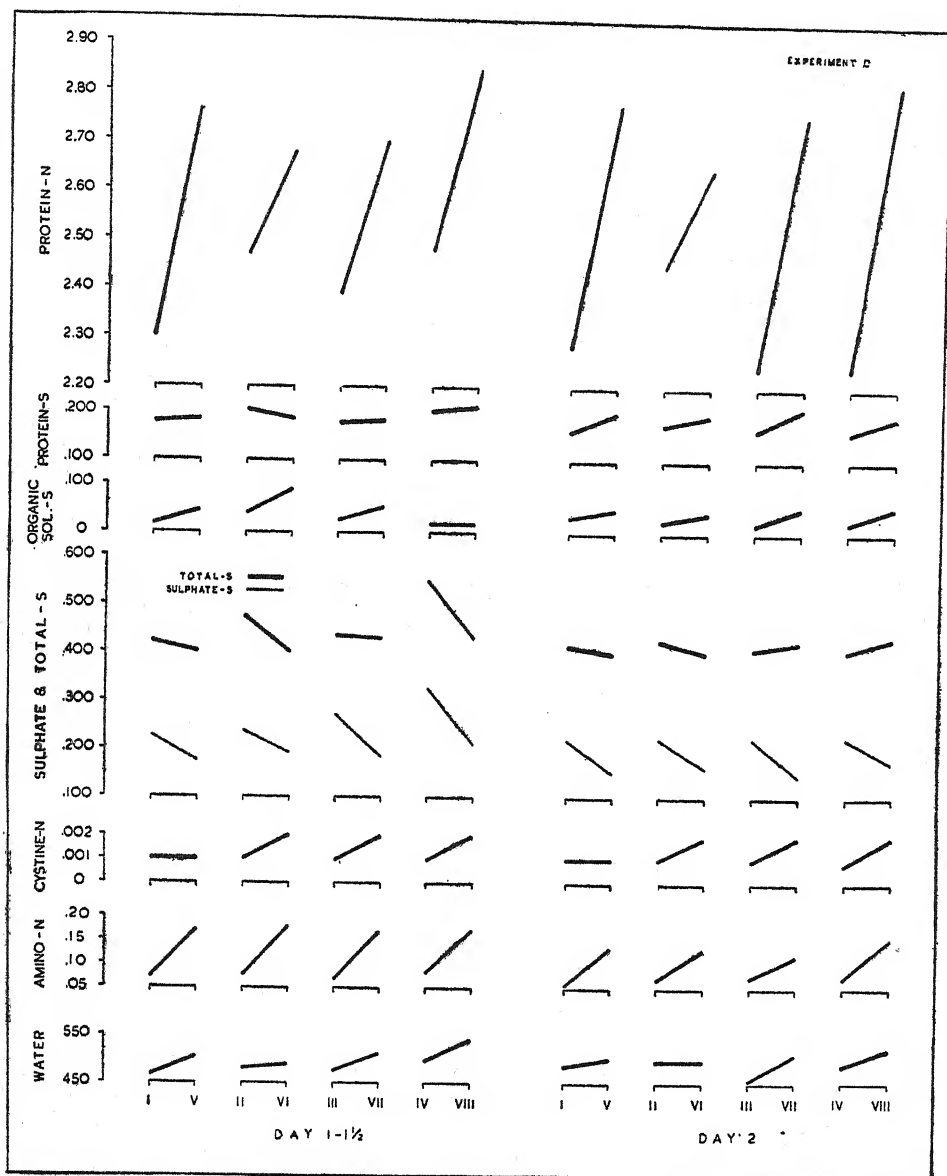


Fig. 5. Effects of treatment with ammonium chloride on the contents of various components of the leaves, Exp. C.

Experiment C. The following treatments were applied in solution 24 hr. after the pots had been placed in the cabinets:

I	0 g. NH_4Cl + 0 g.	cystine per pot
II	0 g. " + 0.125 g.	" "
III	0 g. " + 0.250 g.	" "
IV	0 g. " + 0.500 g.	" "
V	2 g. " + 0 g.	" "
VI	2 g. " + 0.125 g.	" "
VII	2 g. " + 0.250 g.	" "
VIII	2 g. " + 0.500 g.	" "

Pots designated "day 1" and "day 2" were placed in the cabinets on 30 August 1936 and differential treatments were applied on 31 August. The "day 1" pots were removed for analysis on 1 September, 24 hr. after application of the treatments, and the "day 2" pots on 2 September, 48 hr. after application of treatments. Pots designated "day 1½" entered the cabinets on 1 September, received differential treatments the same night and were removed for analysis on 3 September, 36 hr after application of treatments. Four pots per treatment were used for analysis. The results are given in Tables IV and VI and Figs. 3-5.

DISCUSSION OF RESULTS

The dry-weight data

The mean value of the dry weight of the leaves per pot, together with the results of analysis of variance, are presented in Tables III and IV.

In each experiment neither time nor treatment has had a significant effect on the dry weight. The value of such data has been discussed previously by Petrie & Wood (1938*a*). The data afford a guide to the homogeneity of the experimental material. It is not legitimate to draw conclusions among metabolites, the amounts of which are expressed on a dry-weight basis if the basis of expression is shifting, but analysis shows that it is not possible to demonstrate any shift in the dry-weight data presented here.

Experiment A.

This was a small preliminary experiment of an exploratory kind in which three different treatments of potassium sulphate were applied in solution to the plants. The data indicate that the only appreciable increase is in the content of sulphate sulphur. Fluctuations in amounts of other fractions, including protein sulphur and cystine, lie within the limits of error of the analytical methods employed. A more extensive experiment upon similar lines was subsequently planned (Exp. B).

Experiment B.

This experiment was designed to investigate the effects of addition to the sand of different amounts of potassium sulphate on the amounts of nitrogen and sulphur-containing fractions in the leaves.

Time drifts. These are plotted in Fig. 1 and can be considered between days

1 and 2. Two sets of values are available for day 2, designated "day 2a" and "day 2b". On the whole the metabolic components—protein nitrogen, protein sulphur, amino nitrogen, cystine nitrogen, sulphate sulphur, and total sulphur—tend to drift in the same direction as the water content. It will be noted generally that increase in all fractions is greater between day 1 and day 2b than between day 1 and day 2a. The greater differences on day 2b are probably accounted for by mechanical breakdown on that day of a fan which formed part of the circulatory system in the cabinet. This caused the humidity in the cabinet to rise and presumably decreased the rate of transpiration.

Treatment effects. These are illustrated in Fig. 2. Appreciable differences between treatments on each day are apparent only in the case of sulphate sulphur and consequently in total sulphur, i.e. in the sulphur fraction added in varying amounts to the sand. Fluctuations in protein nitrogen are correlated with fluctuations in water content. Amino nitrogen and cystine nitrogen also tend to be correlated with water content and with protein nitrogen, but the variations in amount on any one day are so small as to be within the limits of variation of the method of estimation.

There is a tendency for the drifts in protein sulphur to follow the trend of the drifts in sulphate sulphur, although the fluctuations in amount of protein sulphur are small compared with the changes in sulphate sulphur. Analysis of variance of the duplicate values for protein sulphur showed that time, treatment and interaction were insignificant. The fluctuations in amount of protein sulphur therefore lie within the limits of error of the analytical procedure, and no detectable increase in protein sulphur occurs with increase in sulphate sulphur.

Statistical treatments. The data have been examined as a whole to see whether any common relationships hold among them. Regression functions for proteins on variables are summarized in equations (1), (2) and (3), Table VII. As in the experiments described elsewhere by Petrie & Wood (1938*a, b*) and Wood & Petrie (1938) the equations indicate that the amount of protein increases both with amino-acid content and with water content. In equation (1), however, the coefficient for the amino-acid term is insignificant, although its inclusion improves the fit of the regression. The protein content may be described either in terms of amino nitrogen alone or of water content alone (equations (2) and (3), Table VII). Amino nitrogen and water content are positively correlated, so that in equation (1) the effect of amino nitrogen may be obscured by the effect of water content. If U , the amount of water present in the leaves expressed on a dry-weight basis, be taken as a measure of u , the amount of water actually present in the cytoplasm, then P/U may be a measure of the protein concentration, where P is the amount of protein nitrogen expressed on a dry-weight basis. The mean value of P/U is 475 and the standard error ± 10.4 . The maximum divergences from the mean value are not significant.

Protein nitrogen is positively correlated with protein sulphur ($r=0.6051$; $P^1 < 0.05$), a fact which is also shown by the approximate constancy of the ratio of

¹ P is the probability that a given correlation coefficient should arise, by random sampling, from an uncorrelated population.

Table VII. *Regression equations*

All the regressions are significant at or below the 5 % point. Coefficients significant at or below the 5 % point are given in bold figures, those insignificant in *italic* figures.

Notation

P =protein nitrogen; A =total amino nitrogen; U =water content.

V =percentage of variance of the dependent variable ascribable to the average effect of the independent variables.

D.F.=residual degrees of freedom.

$\sqrt{(\text{res. var.})}$ =square root of variance of dependent variables not accounted for by the regression.

	V	D.F.	$\sqrt{(\text{res. var.})}$
Exp. B. (1) $P=a+b_1A+b_2U$ $a=0.787$ $b_1=3.363 \pm 2.484$ $b_2=0.00268 \pm 0.00120$	77.3	9	0.551
(2) $P=a+b_1A$ $a=1.758$ $b_1=8.101 \pm 1.370$	69.35	10	0.064
(3) $P=a+b_1U$ $a=0.363$ $b_1=0.00407 \pm 0.00066$	75.82	10	0.057
Exp. C. (4) $P=a+b_1A+b_2U$ $a=0.794$ $b_1=3.553 \pm 0.547$ $b_2=0.00271 \pm 0.00114$	85.19	13	0.083

these two quantities (Table V). Discussion of this ratio is reserved for a later section.

Regression equations for protein sulphur on amino nitrogen and water content have not been calculated, since it is apparent that the same relations will hold here as for protein nitrogen.

Conclusions. Provided other conditions remain constant, applications of increasing amounts of sulphate to plants brings about an increase in sulphate sulphur in the leaves, but there is no detectable increase in protein sulphur, cystine sulphur or soluble organic sulphur. Increase in water content is associated with an increase in protein sulphur and protein nitrogen as well as in other sulphur and nitrogen fractions.

Experiment C.

In this experiment the amino-acid cystine was applied to plants in four different treatments at two different external nitrogen concentrations.

Time drifts. These are illustrated in Fig. 3. In treatments I-IV, where additional cystine but no extra ammonium salt was added, the drifts in nitrogen fractions are small, but sulphate sulphur, protein sulphur and total sulphur decrease in amount. It is apparent that transport of sulphur compounds has taken place from the leaves. In treatments V-VIII, where similar amounts of cystine were added but at a higher nitrogen level, total sulphur remains approximately constant in amount, and drifts in other fractions are small. This may indicate that a steady state had been approached by day 1½.

In treatments I-IV it is notable that decrease in amount of sulphate sulphur between days 1 and 2 is greater the higher the cystine treatment. It is possible that

the initial increase in sulphate sulphur on day 1 is due to absorption and oxidation of cystine, the amount of which remains approximately constant. The ultimate fall in sulphate sulphur on day 2 may be ascribed to gradual adjustment of the internal sulphate concentration with that present in other tissues and in the sand since by day 2 the value has fallen to approximately the same level in each of treatments I-IV.

In treatments V-VIII decrease in sulphate sulphur occurs between days 1½ and 2, but this is accompanied by increase in protein sulphur, the total sulphur remaining constant in amount on the two days.

Treatment effects. (a) Cystine treatments. The effects of these are illustrated in Fig. 4. There is no appreciable change with treatment in any fraction except in the case of sulphate sulphur and total sulphur on day 1. The loss of total sulphur from the leaves between days 1 and 2 probably indicates that the sulphur system in the leaves had not approached a steady state on this day. However, the increase in sulphate sulphur affords evidence that cystine actually entered the leaves, since the added cystine provides the only source of additional sulphate to the extent found. It is probable therefore that cystine absorbed by the plant was rapidly oxidized to sulphate but had no other detectable effect upon the amounts of other metabolic components.

(b) Ammonium treatments. These are illustrated in Fig. 5. Since a loss of total-sulphur compounds has occurred from the leaves on day 1 comparisons have been made between treatments on day 2 only. In effect, since it has been shown that increasing amounts of cystine had no discernible effect upon the amounts of other metabolites, each treatment pair in Fig. 5 is a replication.

Increase in the external concentration of ammonium salt has increased protein nitrogen, amino nitrogen and water in the manner described by Petrie & Wood (1938*a*). Treatment has also caused an increase in protein sulphur and, except in two instances, in cystine also. Since the total sulphur remains approximately constant in amount it is probable that the increase in protein sulphur and in cystine has occurred at the expense of sulphate sulphur which decreases in amount with treatment.

It should also be pointed out that the organic soluble sulphur increases with increased ammonium treatment. The values for organic soluble sulphur are not very reliable, since they are obtained by difference of two values of similar magnitude, viz. sulphate sulphur and total soluble sulphur; nevertheless, the values obtained in the case of the increased ammonium treatments are consistently higher than in untreated plants (Table VI). The organic soluble sulphur will include methionine which, according to Pollard & Chibnall (1934), is present in fairly large amounts in grass proteins.

Statistical treatment. The regression of protein nitrogen on amino nitrogen and water content is given in equation (4) (Table VIII). The regression accounts for 85% of the total variance and indicates that the relation between protein nitrogen and these variables is of the same type as that discussed by Petrie & Wood (1938*a, b*).

There is a high positive correlation between protein nitrogen and protein sulphur ($r=0.7279$; $P<0.01$). Discussion of the relation of protein nitrogen to protein

sulphide is reserved for a later section. Regressions for protein sulphur on amino nitrogen and water content have not been calculated but are clearly of the same type as that for protein nitrogen.

The decrease in sulphate sulphur which accompanies increase in protein sulphur and amino nitrogen may be expressed as correlation coefficients. The correlation between sulphate sulphur and amino nitrogen is highly significant ($r = -0.6643$; $P < 0.01$). A high negative correlation also occurs between protein sulphur and sulphate sulphur on day 2 ($r = -0.9440$; $P = 0.02$).

Conclusions. The data of Exp. C indicate that application of cystine to the sand in which plants are growing causes no appreciable increase in protein sulphur or in other organic sulphur fractions, but cystine is apparently absorbed and oxidized to inorganic sulphate. Supply of additional nitrogen as an ammonium salt in an amount unaccompanied by a fall in the water content of the leaves (cf. Petrie & Wood, 1938a) brings about an increase in the amounts of protein sulphur and cystine sulphur whilst the sulphate sulphur decreases in amount; the content of total sulphur is not appreciably affected by this treatment.

Approach to steady state

The argument that a drifting steady state was approached rests partly upon the assumption that when the drift of one component, e.g. water content or amino-acid, changed in direction the lag in the drift of other components, e.g. protein, was not great.

In Exp. B, between day 1 and day 2a the amounts of all reactants in the system, except sulphate sulphur in treatments III and IV, did not change appreciably (Fig. 1). On day 2b increase in water content is accompanied by increase in amounts of all reactants. It is possible therefore that the system was not far removed from a steady state. In Exp. C the changes in amount of reactants are small in most cases between days 1 and 2; in treatments I-IV protein sulphur decreases between days 1 and 2, but when extra ammonium salt is added in treatments V-VIII the protein sulphur increases in amount.

The goodness of fit of the regression functions for protein nitrogen would be accounted for if this system were near a steady state during the experiments.

GENERAL CONCLUSIONS

It would be premature to discuss fully the probable sequence of reactions making up the sulphur metabolism of the plant until further data from experiments in progress are completed; nevertheless, certain tentative conclusions may be drawn from the results of the experiments described here.

The relation of protein nitrogen to protein sulphur

In Exps. B and C there is a significant positive correlation between protein nitrogen and protein sulphur. The values for the ratio of protein nitrogen to protein sulphur are given in Tables V and VI

It is important to establish whether more than one protein occurs in the cytoplasm or whether the protein is of variable constitution. Miller (1923) analysed

plants of red clover at intervals during a growing period of 5 months. He determined the nitrogen and sulphur in that portion of the plant extracts which were precipitated by acetic acid and which would therefore consist chiefly of proteins. He states that in this fraction the ratio of nitrogen to sulphur remained approximately the same regardless of the stage of development of the plants.

Lugg (1938) has analysed pure proteins extracted from the leaves of such diverse plants as *Phalaris tuberosa*, *Dactylis glomeratum*, *Trifolium subterraneum* and *Atriplex nummularium*. He found that the ratio of nitrogen to sulphur was the same in each protein, and his preliminary results suggest that probably only one albumin and one globulin are present in the cytoplasm.

The data presented here are insufficient to decide whether protein is constant or variable in its sulphur content during treatment. The value of the ratio of protein nitrogen to protein sulphur is approximately constant in Exp. B. In Exp. C, except for the values for day 1, and in spite of fairly wide fluctuations in protein content brought about by treatment, the value for the ratio remains approximately constant. The lower values for the ratio on day 1 suggest, however, that the protein is variable in constitution and that the nitrogen fractions may approach a steady state more rapidly than do the sulphur fractions.

In neither experiment is there a wide range of protein content. The data of the experiments described here show that on the whole there is an approximate constancy in the ratio of protein nitrogen to protein sulphur, but all that they demonstrate with certainty is that there is a high positive correlation between the two quantities. Experiments are in progress to determine the value of the ratio at different stages of the life cycle and also during starvation.

The position of cystine in the system

The amount of free cystine in the tissues is small whatever the treatment applied in our experiments. Nightingale *et al.* (1932), working with tomatoes, noticed that even during proteolysis "little if any sulphydryl sulphur is formed". It might be expected, since cystine is an easily oxidizable compound, that the equilibrium between sulphate and cystine is towards the side of complete oxidation.

It is a well-established fact that amino-acids are oxidized to ammonia and carbon residues and also in the case of cystine, as shown in our experiments, to sulphate. Furthermore, a reversible relation holds between proteins and amino-acids which is dependent on the water content (Petrie & Wood, 1938*a, b*).

In the experiments described here in which the water content did not decrease as the ammonium concentration increased, the amount of cystine increases as the other amino-acids increase in amount. Accompanying this increase in amino-acids is an increase in protein nitrogen and protein sulphur. It would appear therefore that in so far as cystine is related to protein, the schema set out in the second section of this paper is in accord with the experimental data and may be formulated



where C = cystine, A_r = amino-acids other than cystine, P = protein.

If this relation holds it might be expected that increase in cystine concentration might lead to a decrease in that of the other amino-acids and to an increase in protein. Such an effect was not discernible in Exp. C. It is possible that the analytical methods were not sufficiently refined to detect small changes in amounts of the sulphur components or that some other amino-acid is "limiting", but the evidence of day 1 in this experiment suggests that the absorbed cystine was oxidized to sulphate; thereafter the sulphate had time to diffuse back to the stems.

The relations between sulphate sulphur and cystine and protein sulphur

The data of Exp. A show that an increase in amount of sulphate sulphur does not bring about increase in any organic sulphur fraction. This condition is different from that which occurs in cases of sulphur deficiency where sulphate concentration limits the growth processes and causes definite pathological symptoms in the leaves. In such cases addition of sulphate causes an increase in protein sulphur as described by Eaton (1935), Nightingale *et al.* (1932).

The results of Exp. C show that when the external concentration of ammonium salt is raised one step there is an increase in cystine and in protein sulphur, as well as in nitrogen fractions, accompanied by a decrease in sulphate sulphur. It is apparent that a similar raising of the nitrogen level is responsible for the increase in sulphur and nitrogen fractions found on different days in Exp. B. In the latter experiment, although increasing amounts of sulphate brought about no alteration in the sulphur or nitrogen fractions on any one day, the water content of the plants increased on day 2*b* and was accompanied by an increase in organic sulphur and organic nitrogen fractions. It is assumed that increase in water content caused a decrease in concentration of all cell metabolites, including ammonium salts; there would therefore be a disturbance of the balance holding between internal and external concentrations of ammonium salts and some of the salt would tend to enter the plant in order to reapproach steady state conditions. This would result in increased synthesis and consequent increase in sulphur and nitrogen fractions expressed on a dry-weight basis.

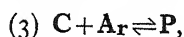
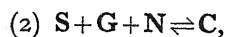
It is also possible that some of the increase in water content on day 2*b* can be accounted for as water held by imbibition by the increased amount of protein present (cf. Petrie & Wood, 1938*b*). It is more probable, however, that the increase in water content on this day was brought about by decreased transpiration consequent to the higher humidity resulting from breakdown of the fan.

The picture suggested by the experiments described here is that ammonia nitrogen is acting as a limiting factor: that is to say, at a steady state increase in the internal concentration of sulphate sulphur at any particular nitrogen concentration does not bring about an increase in the protein sulphur concentration; the latter increases however if the ammonia nitrogen concentration is increased.

The intimate connexion between sulphur metabolism and nitrogen metabolism had been noticed by Miller (1923) who stated "that with clover growing in sand cultures it is possible by reducing the available nitrate, not only to limit growth and

nitrogen content but also to decrease the sulphur assimilation; the amount of sulphur taken up by the plant is limited by the total nitrogen absorbed".

In attempting to interpret this phenomenon we may turn first to the hypothetical scheme formulated in the second section of this paper and consider the problem from the viewpoint of the Law of Mass Action. We may write this scheme:



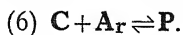
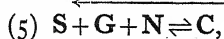
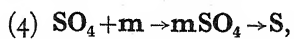
where SO_4 represents sulphate and S sulphide ions respectively, G = the glycolytic products, N = ammonia, C = cystine, A_r = amino-acids other than cystine, and P = protein.

In this series of balanced actions, the equilibrium point in equations (1) and (2) might be expected to be well over to the left-hand side as suggested by their free energy decrease.

If, at any time, under a given set of conditions we imagine this system to have attained a steady state, then an increase in ammonia concentration would bring about a decrease in sulphide concentration and an increase in the concentrations of cystine and of protein. The rate of oxidation of sulphide would therefore decrease, and equilibrium in reaction (1) would not be attained until the sulphate concentration had fallen to a proportionate extent.

But the simple scheme formulated above is also rendered untenable by the fact that an increase in sulphate concentration would be expected to bring about, at the steady state, an increase in sulphide concentration and a consequent decrease in ammonia concentration and an increase in that of cystine and protein sulphur. It is clear from Experiment A that this does not take place.

It would appear more likely therefore that the sulphate ion enters into intermediate compound formation with some other constituent of the cell and that it is this compound which is reduced to sulphide. It has been pointed out in the second section of this paper that the mechanism of sulphate reduction is quite unknown; "compound formation" therefore must be considered in a wide sense. It may be that sulphate combines with some chemical entity in the cell, enzymatic or otherwise, or else that a portion of the sulphate ions absorb energy and become "reactive". Denoting the unknown component by m , the scheme may be formulated:



In this scheme if the concentration of sulphate is small compared with m , as might occur in cases of sulphate deficiency, then, provided N and G are in excess, an increase in concentration of cystine and of protein sulphur might be expected if the sulphate concentration is increased. If, on the other hand, the sulphate

concentration is large compared with *m*, then cystine and protein sulphur would be related to *m* and not to the total sulphate.

If *m* were an enzyme or if the reduction of sulphate were photosynthetic, then it might be expected to remain constant in amount with varying treatment. Under these circumstances the concentration of sulphide would also remain constant. This is feasible, since the differences between the Folin-Marenzi and Sullivan methods suggests that the amount of sulphides other than cystine is very small. If the amount of sulphide does remain constant then an increase in ammonia would be expected to bring about an increase in cystine and in protein sulphur accompanied by a decrease in sulphate sulphur, such as is shown in the experiments described here.

In the foregoing arguments it has been assumed that amino-acids are precursors in the synthesis of protein. The alternative view that proteins are formed *en bloc* from glycolytic products, ammonia and sulphide, and that amino-acids, including cystine, are derived solely from protein is also in accord with the observed facts, so that from the data presented here it is not possible to distinguish between the alternative hypotheses.

SUMMARY

This paper describes an investigation into the relations that existed in the leaves of plants among the amounts of sulphur compounds when the plants were subjected to different treatments under certain constant environmental conditions. The treatments consisted in varying dosages of potassium sulphate, ammonium chloride and cystine applied externally to the plant. The following conclusions are drawn and there is reason to believe that these apply to the steady state, though such state was not necessarily attained in our experiments:

(1) Increase in sulphate sulphur content under the conditions described here does not increase the content of cystine or of protein sulphur.

(2) Increase in ammonia nitrogen increases the contents of cystine and protein sulphur as well as that of amino nitrogen and protein nitrogen. This is accompanied by a decrease in sulphate sulphur.

(3) The addition of cystine to the sand containing the plants brought about an initial increase in sulphate sulphur but brought about no change in the cystine or protein sulphur contents.

(4) In the experiments described here the ratio of protein nitrogen/sulphur remains approximately constant.

(5) The picture suggested is that ammonia nitrogen acts as a limiting factor in the formation of protein sulphur from sulphate, and a schema is presented for the probable sequence of reactions in sulphur metabolism.

(6) Analytical methods for the determination of various sulphur fractions are described.

The authors are indebted to Dr A. H. K. Petrie of the Waite Institute, University of Adelaide, who placed the facilities of his laboratory at our disposal on harvest days; and to Mr G. E. Briggs, F.R.S., of St John's College, Cambridge, with whom one of us discussed some of the results reported here.

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OBSERVATIONS ON THE ANATOMY OF THE CRICKET-BAT WILLOW (*SALIX CAERULEA* SM.)

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(With 3 figures in the text)

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I. INTRODUCTION

IN the course of work on the watermark disease of the cricket-bat willow *Salix caerulea* Sm. it has been necessary to make a detailed study of the structure of the secondary wood. So far as could be ascertained, no detailed description of this wood has been published. Comparisons of the elements of the wood of various species of *Salix* have been given by Penhallow (1905) and Herrmann (1922), but these were of limited scope.

During this investigation attention has been focused upon the relationship of the leaf trace to the diffuse-porous structure of the wood, and upon the anatomical relationship of a branch to the main stem which bears it. These examinations have been carried out on branches from one to five years old, as in such young branches there may be complications in the structure of the stem-branch crotch.

2. THE STRUCTURE OF THE WOOD

(a) *The primary wood*

Any transverse section through an internode of a one-year-old branch shows a central pith, usually pentagonal in shape, made up of isodiametric parenchymatous cells. There is a discrete endarch leaf-trace bundle (primary bundle) at each angle of

the pith. These bundles are markedly fan-shaped and consist of from six to twelve rows of vessels. Each row of vessels contains about six contiguous units with spirally thickened walls; neighbouring rows of vessels are separated by a single radial row of parenchyma cells. These parenchyma cells are almost square in transverse section; their depth is three or four times greater than the breadth, and the end walls are horizontal. Simple pits occur on the tangential and transverse walls.

Completely surrounding the pith and separating it from the wood is a zone of thick-walled parenchyma cells which are similar in shape and size to the parenchyma cells of the bundle rays. This thick-walled parenchymatous tissue exists as a layer only one or two cells wide radially along the sides of the pith column, but at the angles of the pith, between the leaf-trace bundle and the pith, is a crescent-shaped, compact mass of parenchyma.

There is at least one leaf-trace bundle on each "side" of the pith column; these bundles resemble the angle bundles in construction except that they are not fan-shaped and are separated from the pith by only one or two rows of parenchymatous cells.

(b) *Secondary wood*

(i) *The vessels.* The secondary wood is diffuse-porous. In a transverse section (of mature wood) the vessels are numerous or very numerous (Chattaway, 1932), medium sized (Chalk, 1938) and are without definite pattern. The number of vessels per unit area is slightly greater in the outer part of the ring than in the early wood but the vessels are larger in the early wood and may occupy a greater total area.

The vessel elements are medium sized (*Tropical Woods*, 1937); the end walls are moderately oblique and each contains a large oval simple perforation, the remainder of the end wall being covered with bordered pits if the wall is sufficiently oblique. The intervacular pit-pairs are moderately numerous, large ($8.5\ \mu$ in diameter), and alternate. The borders are hexagonal in outline, and the apertures oval and horizontal. The pits to the ray cells are described with the ray cells.

In any transverse section there is a limited number of pore multiples—groups of two or three radially contiguous vessels—and a few irregular groups. Owing to the obliqueness of the end walls of some elements, a single vessel may (if the section passes through the end wall) appear in transverse section as a radial pair or even as a triad if the end walls of the elements are very oblique.

In the wood on the outer boundary of the annual ring small vessels occur, these are described later.

(ii) *Fibres.* The fibres are typically hexagonal in transverse section, are arranged in regular radial rows, are moderately short (*Tropical Woods*, 1937) and are thin walled, the double cell wall of adjacent fibres being less than one quarter the diameter of the fibre lumen. Pits are very small and simple, and most numerous on the radial walls, rather irregularly distributed, sometimes in transverse pairs. In young wood the fibres may contain starch grains.

(iii) *Wood parenchyma.* Wood parenchyma occurs as a complete ring separating the wood of two successive annual rings, but is absent from the rest of the wood except for very occasional cells associated with a vessel. The ring of wood paren-

chyma cells corresponds in position with "terminal" parenchyma, but as it is not present between the cambium and the secondary wood in winter, it must be formed at the start of the season's growth. Chowdhury (1936) has shown that in *Terminalia tomentosa* the "terminal" parenchyma differentiates at the beginning, and not the end, of the season, and has suggested the term *initial parenchyma* as more logical.

(iv) *Rays*. The rays of various species of *Salix* have been described by Holden (1912), by Herrmann (1922), and by Penhallow (1905), who has drawn up a key to the species based entirely on the rays. The rays are uniseriate, very fine (Chalk, 1938), very numerous and very low (Chattaway, 1932), and heterogeneous. They usually consist mainly of procumbent cells, the upright cells being limited to a single row at the top and the bottom. Occasionally two rows of upright cells may be present on the margins, and rows of upright cells may also occur among the procumbent cells. The upright cells are distinctly higher axially than the procumbent cells and narrower radially; they can be distinguished in tangential sections. Multi-seriate rays are present below a leaf trace.

The pit-pairs between ray cells are simple; there are no pits between the ray cells and the fibres, nor between the procumbent cells and the vessels. The wall between an upright cell and a vessel is covered by conspicuous and apparently simple pit-pairs which are large and hexagonal or round in shape. Under high magnification it is evident that there is a very narrow border in both the vessel and ray pits.

(v) *The outer boundary of the annual ring*. In this region the wood elements differ from those in the rest of the annual ring. The vessels, as seen in transverse section, are angular—often square—in shape, and are very small (Chalk, 1938). Often they occur four together, forming a square. The end walls are usually oblique and bear bordered pits, the simple perforation being usually towards one end of the perforation plate. Generally the perforation can be seen only after maceration; in sections the narrow vessel elements resemble tracheids. Wood parenchyma may be present, either as isolated cells or in groups of two or three cells, usually associated with a vessel.

3. THE NODAL REGION IN A YOUNG STEM

(a) *The leaf-trace system*

Three bundles enter the stem from every leaf. The central bundle, on leaving the petiole, passes vertically downwards through the cortex for a distance of about 5 mm. and then passes almost horizontally through the wood, curving slowly downwards as it approaches the pith until finally it assumes a vertical path along an angle of the pith column. In the inner part of the wood the angle between the pith column and the bundle is occupied by a radial proliferation of the pith. As the inner ends of the rays are always at right angles to the pith the ray system is distorted and appears fan-shaped in transverse section. The wood elements from below are displaced to the sides, but re-establish continuity on the upper surface of the trace.

Each of the two lateral bundles, on leaving the petiole, takes a tangential and downward path through the cortex, girdling the stem through an angle of about 70° before entering the wood. In the wood its course is similar to that of the central bundle.

(b) *The bud*

Immediately above the point of departure of the leaf-trace bundle—often before the pith arm has been completely replaced by the wood elements—a narrow pith column passes outwards through the wood to the base of the bud. This is not accompanied by any distortion of the wood ray system. The wood elements from below, whose continuity above the leaf trace has been recently re-established, terminate on its lower surface and continuity is re-established on its upper surface by the bending in of elements from the sides.

Buds in the axils of the two lowest leaf rudiments of the axillary bud are well developed before the rest of the axillary bud has reached an advanced stage of differentiation. These two small buds are the "accessory buds" characteristic of the node in all willow species.

Frequently secondary shoots do not develop on a stem until the third or fourth year of its growth. Until the bud develops there is a narrow pith column connecting the base of the bud to the pith of the stem.

4. THE RELATION BETWEEN LEAF TRACE AND WOOD STRUCTURE

Priestley & Scott (1936) suggest that in ring-porous types only the large vessels in the early wood are in connexion with the leaf traces, but that in diffuse-porous types, when the traces of higher inserted leaves lose their identity, the vessels become scattered through the wood, thus giving the wood its characteristic structure. This is true of the diffuse-porous willow wood.

The course of the vessels of leaf-trace bundles was followed in a young shoot of willow by injecting the topmost petiole with a suitable dye and cutting serial sections through several internodes below that leaf.

A transverse section through the stem above the first fully developed leaf showed a mass of parenchymatous tissue in which the vessels were differentiating. Three fully developed leaf-trace bundles entered the stem from the first leaf, and in the internode below the first leaf these occupied adjacent angles of the well-defined pentagonal pith (Fig. 1 (i)). Other wood elements had not been differentiated. At the second node three more leaf-trace bundles entered the stem; in the internode below the second leaf the pith column had a changed shape, being re-orientated so that one of the bundles from the first leaf lay along one side of the pentagon, the remaining three angles being occupied by the three bundles from the second leaf (Fig. 1 (ii)). In this internode a narrow zone of fibres had differentiated around the pith, but the only vessels present were those of the leaf-trace bundles. In the third internode the pith was again re-orientated and the three bundles from the first leaf now occupied three sides of the pentagon.

In the internode below the seventh leaf more secondary xylem was differentiated and in addition to the leaf-trace bundles around the pith, other vessels were present

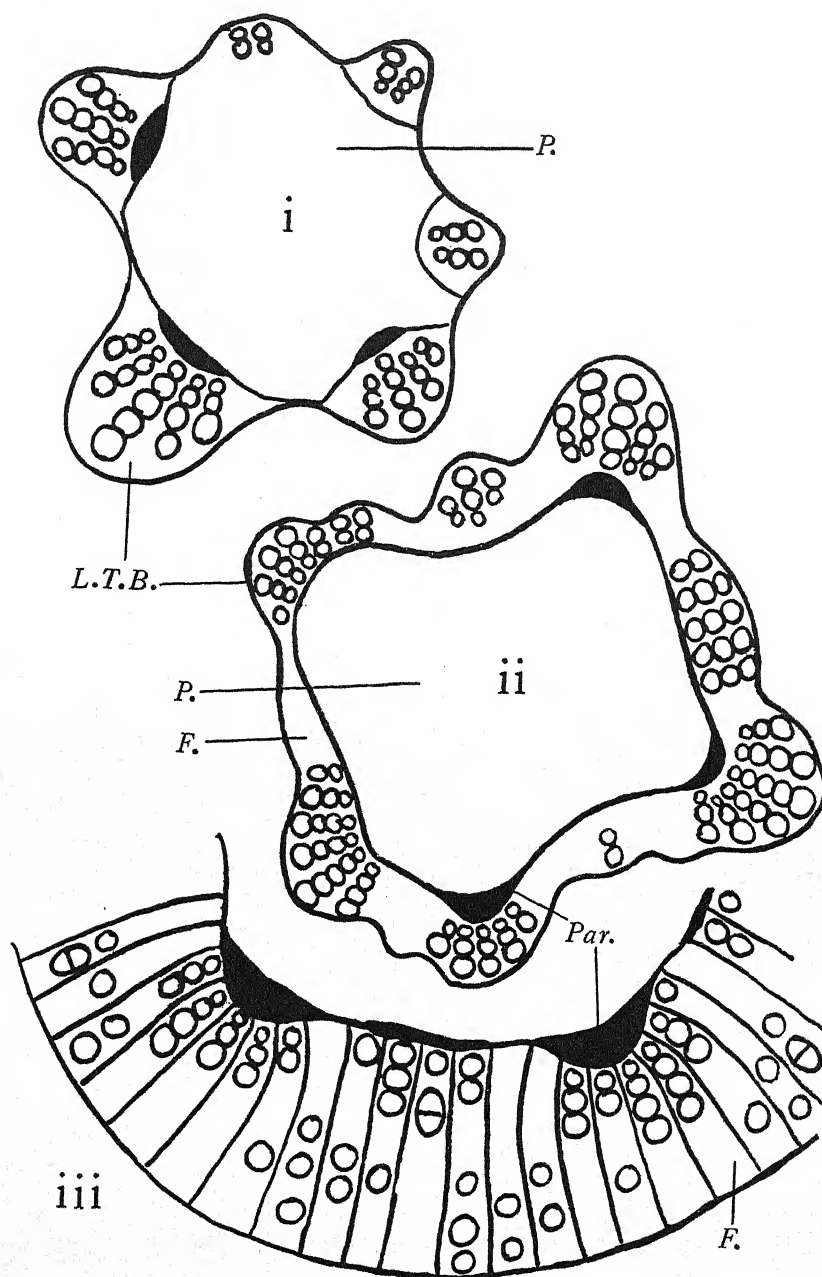


Fig. 1. (i) Section through the internode below the first leaf. The wood is made up of leaf-trace bundles. (ii) The second internode. A thin zone of fibres has been formed. The only vessels are those of the leaf-trace bundles. (iii) The seventh internode. In addition to leaf-trace bundles, diffuse groups of vessels are present in the outer wood. The phloem and cortex are not shown. *P.* pith; *Par.* mass of small celled parenchyma; *F.* fibres; *L.T.B.* leaf-trace bundle.

in the outermost wood. Careful examination showed that these apparently scattered vessels in the outermost wood were really aggregated in diffuse groups (Fig. 1 (iii)), and the stained vessel walls in three of the groups indicated that each of these three groups of vessels was the downward continuation of a leaf-trace bundle from the first leaf. Fusion of adjacent vessels had occurred in the intervening internodes so that the total number of vessels in each trace had been reduced. In the eighth internode the vessels with stained walls were no longer aggregated in groups but were scattered without pattern throughout a small peripheral strip of wood.

Thus when a leaf trace enters the wood it runs down the edge of the pith column for several internodes before it loses its identity; when this happens the vessels become scattered throughout the secondary wood. In any transverse section through a woody stem the vessels near the periphery of the secondary wood are in direct connexion with the leaf-trace bundles of the upper leaves; when the shoot lengthens and new leaves unfold, the new vessels which are formed during the increase in girth in the lower parts of the shoot are in connexion with them.

5. THE RELATIONSHIP BETWEEN BRANCH AND STEM

The path which the vessels take in the crotch has been studied in a naturally injected four-year-old crotch. In the watermark disease of willows the bacterial pathogen (*Bacterium salicis* Day) is found chiefly in the vessels (Day, 1924). When a diseased branch is cut the contents of the vessels turn brown. It is thus possible to find material in which the path of the vessels is shown by continuous brown streaks. This property of diseased wood is invaluable, as it is extremely difficult to inject mature wood satisfactorily with the usual dyes.

On the underside of the junction the vessels of the branch are continuous with those of the main stem below the branch, but on the upper surface of the branch the vessels run down into the crotch, where they turn almost at right angles and run straight down the sides of the stem, as shown in Fig. 3. The vessels of the main stem on the side facing the branch take a similar course; there is thus no direct continuity between the vessels on the upper surface of the branch and those on the side of the stem facing it, only a lateral contiguity. The vessels on the sides of the branch take a course midway between the extremes.

The wood of the branch is continued below the crotch as a sector in the circle of the wood of the main stem (Fig. 2 (a)); the sector "fits in" to a corresponding gap in the stem wood, and the only mechanical union between the two lies in the lateral fusion of the elements along the radial edges of the sector with those along the corresponding edges of the stem wood. This fact accounts for the crotch being mechanically so weak that the branch can be torn from the stem, the splitting taking place *along* the grain and nowhere *across* the grain.

As the branches grow older a large proportion of the smaller side branches become more or less static, and increase in thickness is slow. The main branches thicken more rapidly, and the bases of the small side branches become embedded. In wood about four years old this process often results in a peculiar relationship of

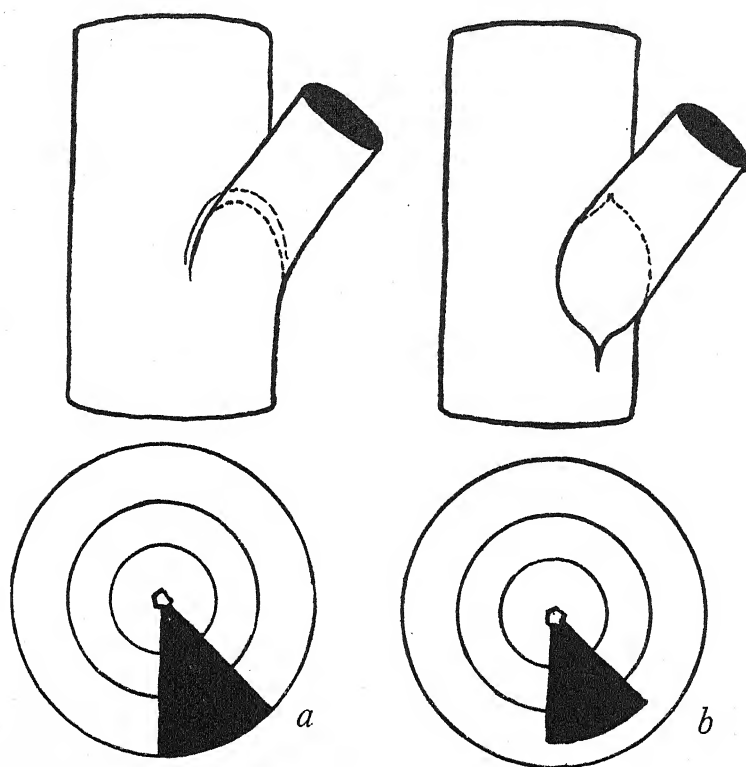


Fig. 2. (a) Normal branch, and section of stem below the crotch showing the sector of branch wood. (b) Invaginated branch, and section of stem below the crotch showing truncated sector of branch wood.

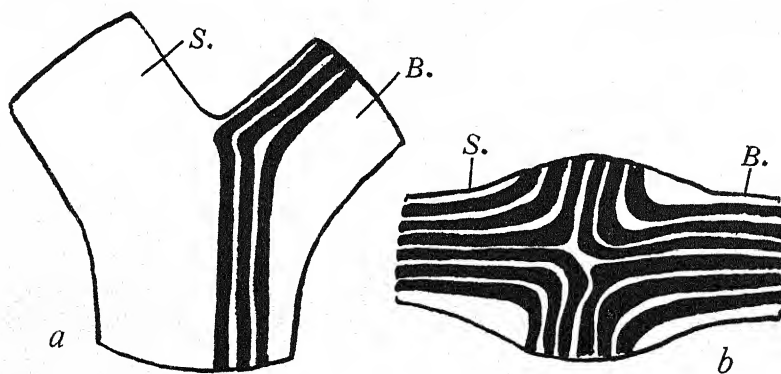


Fig. 3. The black lines show the path of the vessels in a crotch. (a) side view; (b) view from above. S. stem; B. branch.

branch to main stem. Experiments in which dyes were sucked into the wood showed that whereas in the branch the dye had ascended in the whole peripheral circle of late wood, in the stem below the branch it was confined to a sector in the inner part of the annual ring (Fig. 2 (b)). The branch union was dissected and it was found that only the early wood of the stem was in direct connexion with the wood of the branch. Just below the junction the late wood of the stem parted to right and left of the branch, encircling its base in a sheath-like manner, and reuniting above the branch. There was no direct connexion between this late stem wood and the wood of the branch. This late stem wood will be in connexion with branches above the point considered; a large amount of thickening has to occur in the lower parts of the stem to meet the demands of all the growth made in the upper parts of the stem. The smaller branches on the lower parts of the stem cease secondary growth in summer long before the main stem ceases thickening. Thus vessels in the last-formed branch wood will differentiate in continuity with those in the inner part of the annual ring in the main stem. This was confirmed by cutting longitudinal sections.

More branches examined in this way showed that the greater the difference in size and thickness between the stem and branch, the more pronounced was the ensheathing of the base of the branch by wood which was not in connexion with the branch; where the branch and stem were nearly equal in size there was little or no such sheath formed. With further increase in age of such a branch-stem junction, the branch becomes suppressed and its influence on the thickening process in the stem is not so great.

With the growth in length of the branches, the zone in the tree in which these features of anatomical complexity are found moves, every year, farther out from the centre of the tree. The main trunk of the tree, for commercial reasons, is usually kept free from branches to a height of 10 ft.; thickening there is usually regular.

6. SUMMARY

1. A detailed histological description of the wood of *Salix caerulea* Sm. is given. The wood consists of fibres and vessels. Wood parenchyma forms a ring on the inner surface of each year's growth. The wood rays are uniseriate and heterogeneous.

2. The vessels in the diffuse-porous secondary wood of young branches are the downward continuations of the vessels of leaf-trace bundles.

3. In a branch crotch there is no continuity between the vessels of the branch and those of the part of the stem above the crotch.

I am deeply indebted to Dr L. Chalk, of the Imperial Forestry Institute, Oxford, for invaluable help and friendly criticism in the preparation of this paper.

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SOME FEATURES OF THE ORGANIZATION OF THE SPOROPHYTE OF *EQUISETUM ARVENSE* L.

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(With 6 figures in the text)

INTRODUCTION

A REVIEW of the literature on the anatomy and embryology of *Equisetum* reveals certain disagreements between investigators, and furthermore suggests a lack of consistency in the organization of the plant body. As abundant material of young sporelings of *Equisetum arvense* in various stages was available to the present writer it was felt that a critical re-examination of certain phases of the ontogeny of the sporophyte might shed fresh light on the points in dispute. Further, by taking cognizance of the laws underlying the partitioning of cellular masses, it was hoped that a new approach might be made to the study of the organization of embryos such as *E. arvense*.

MATERIAL AND METHOD

The material used in this investigation was partly obtained from a recent road cut with a northern exposure of fine-grained clay kept damp by seepage water. The remainder was grown in the greenhouse from spores. These were sown on similar clay in shallow glass-covered pans watered from below. When antheridia and archegonia had formed, the prothallia were flooded with water once a day to ensure fertilization. For the best growth of the gametophytes it was necessary to keep the cultures quite cool and fairly well shaded in a moist atmosphere.

The fixative used for the prothallia was form-acetic alcohol.¹ After embedding and sectioning the 5-10 μ serial sections were stained with aqueous Cotton Red and Delafield's haematoxylin.

EARLY CELLULAR ORGANIZATION OF THE EMBRYO

As an introduction to the early cellular organization of the embryo, brief reference will be made to the principles of cellular partitioning put forward by D'Arcy Thompson (1917) and which seem to apply to *Equisetum*.

Prof. Thompson shows that theoretically the partitioning of cells obeys the law of minimal surfaces, with the result that only three cells may meet in a point, and consequently in the four-celled stage there must be two triple contacts joined by

¹ 66 % alcohol	90 c.c.
Formalin (Commercial)	5 c.c.
Glacial acetic acid	5 c.c.

a wall, known as the "polar furrow". The length of the polar furrow is greater or less in proportion to the relative magnitudes of the cells in contact, but theoretically is always present. The four-celled and subsequent stages in such partitioning of a discoid cell are reproduced here from Thompson's work (Fig. 1 A-D).

Figures of a number of embryos of *E. arvense* in various stages of development are shown in Fig. 2 A-G. It is significant to observe how closely they conform to the theoretical diagrams referred to above.

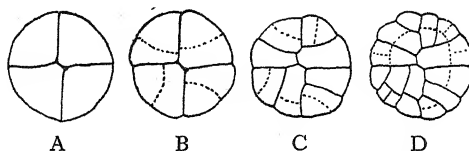


Fig. 1. Theoretical arrangement of successive partitions in a discoid cell. (Redrawn from Thompson.)

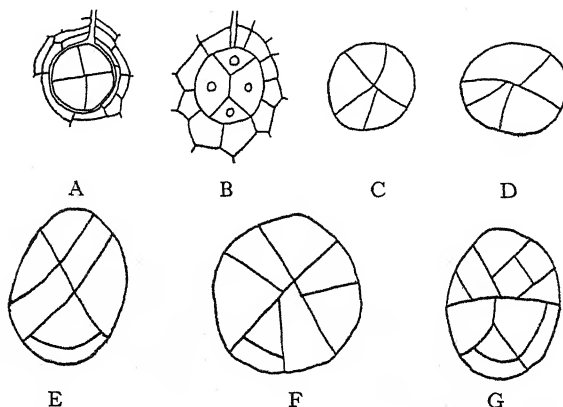


Fig. 2. Stages in the development of the embryo of *E. arvense*. (A-C redrawn from Hofmeister, D-G from Sadebeck, 1878.)

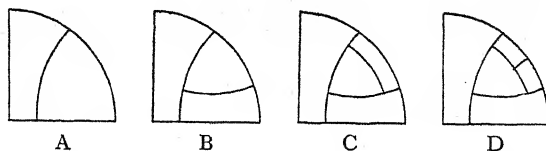


Fig. 3. Theoretical diagrams to show successive partitions in a quadrant. (Redrawn from Thompson.)

The successive theoretical divisions of a discoid quadrant cell are of especial interest. Prof. Thompson (1917) shows that the first division will be by an anticline as illustrated in Fig. 3 A. These anticlinal walls are circular arcs that include a fixed proportion of the quadrantal arc, their length and radius of curvature bearing a constant relation to the radius of the quadrant. This anticline divides the original quadrant into two cells of different shapes, one a triangle with two sides formed of circular arcs and the other a four-sided figure that is approximately oblong. He

states that the least possible partition wall which bisects the area of the oblong will be one more or less at right angles to the long axis of the cell, that is to say a pericline, and that the division of the triangular cell will follow the same pattern as the quadrant, producing again a triangle and an oblong. This and subsequent divisions are illustrated in Fig. 3 B-D. It will be noted that the partitioning of such a quadrant when carried to the stage of Fig. 3 D produces a triangle which theoretically will continue to divide by the formation of walls parallel to its sides and in the same cyclic order.

In the case of a spherical embryo such as that of *Equisetum* it is evident that the partitioning of the three-dimensioned homologue of the discoid quadrant, viz. the octant of a sphere, will again proceed in a similar cyclic sequence. In this case, however, before the periclinal wall develops there will be three, instead of two, anticlinal divisions, each roughly parallel to the plane surfaces of the octant. The formation of the pericline cuts off a tetrahedron which will continue to divide by walls parallel to its four sides. It is important to note that such a tetrahedron has all the morphological characteristics of the apical cell of a root.

Theoretically, if this series of divisions took place uniformly in all the octants of the zygote eight tetrahedrons would result, all of which would have the requisite shape for root initiation. This potentiality of development, however, is never realized since other factors, perhaps those associated with organ determination, modify the continuance of the theoretical location and sequence of the divisions that bring about the formation of the spherical cellular mass.

There is general agreement¹ that the root in *Equisetum* does actually arise from the lower half of the proembryo. In the opinion of the present writer it is unimportant whether it arises from any particular one of the four lower octants since all have equal morphological potentialities. In the majority of cases the root in *E. arvense* is more or less aligned with the major axis of the archegonium and in consequence it is safe to assume that it arises from the hypobasal tetrahedron nearest to this axis. It is to be noted that the required type of tetrahedral apical cell would not arise in all octants until the 48-celled stage, and even in any one octant four divisions would be required to produce a tetrahedron. Nevertheless, some embryologists have considered it possible to label quadrant cells as leaf, stem, foot and root initials.

The exact point of origin of the stem in *Equisetum* seems to be as indefinite as that of the root. The apical cell that gives rise to it may develop in any one of the four upper octants of the proembryo, usually, however, in the one most closely aligned to the major axis of the archegonium.

The development of the stem apex shows clearly the effect of factors modifying the theoretical character and sequence of divisions. This is evidenced by the expected periclinal wall failing to appear after the third anticlinal division of the octant. Nor do periclinal walls appear subsequently in this apical cell. At a certain stage in the development of the proembryo this cell increases in size so that its free surface

¹ Jeffrey (1899) states that in *E. hiemale* the root arises from the epibasal portion of the embryo although, as Campbell (1928a) has pointed out, his figures are capable of a different interpretation.

bulges slightly, destroying the strict sphericity of the cellular mass (Fig. 4A). The subsequent divisions (Fig. 4B) of the apical cell are in accord with the statement made by Thompson (1917, p. 403): "And so the divisions will proceed, by oblique alternate partitions, each one tending to be, at first, perpendicular to that on which it is based and also to the peripheral wall; but all these points of contact soon tending, by reason of the equal tension of the three films or surfaces which meet there, to form angles of 120° . There will always be, in such a case, a single apical cell, of more or less distinctly triangular form."

There is then, in the opinion of the present writer, sufficient evidence to indicate that the laws enunciated by D'Arcy Thompson are applicable to the early organization of the proembryo of *E. arvense* and also to the delimitation of the initials and subsequent development of the adult organs.

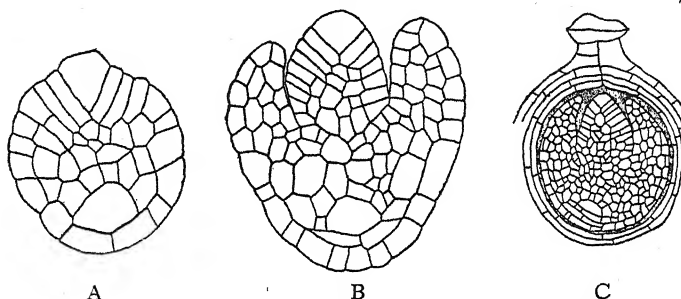


Fig. 4. Later stages in the development of the embryo.
(Redrawn from Sadebeck.)

PROTHALLIAL NUTRITION

In spite of the fact that very little is known of the physiological mechanism of the absorption of nutrients by embryos it is possible, from morphological evidence, to draw certain general conclusions with regard to the region, or regions, in which this absorption takes place. The prothallial nutrition of the young sporophyte falls naturally into three stages, the nutrition of the proembryo, of the embryo proper, and of the sporeling before it attains complete independence from the gametophyte.

The proembryo is completely invested by jacket-like cells (cf. Fig. 2A) and since there is no special area of contact and no differentiation of its cells (*vide* Figs. 2A-G and 4A) it would seem logical to infer that absorption is general over its entire surface.

In the embryo when the adult organs have become initiated (Fig. 4B and C) this generalized type of absorption still appears to be operative, with possibly greater activity in those parts of the embryo that are in more intimate contact with the investing cells, e.g. the base of the first leaves and the lower part of the embryo shown in Fig. 4C.

At a still later stage, when the adult organs have grown beyond the gametophyte, the cells forming the base of the first leaf-sheath, stain more deeply, thus differentiating an absorptive region which is quite comparable in the character and density of

staining of its cells with the condition in bryophytes, where the absorptive character of the cells is unquestioned. In a transverse section through the base of the first leaves in *E. arvense*, an absorptive region can be discerned extending for about three-quarters of the circumference of the sporeling. There is thus formed an haustorial region at the base of the first leaves which suggests a cotyledonary function. This area Campbell (1928b) has designated the foot in the case of *E. debile*, stating that the large foot—constituting the entire hypobasal portion of the embryo—later merges with the base of the first leaf-sheath. As he makes no reference to the absorptive function of the foot, it appears that the area was so designated on purely morphological grounds. It is regrettable that Campbell does not draw attention to the dual significance of the term “foot” which implies both location at the base of the archegonial axis and an absorptive function. Owing to the fact that the presence of morphologically specialized cells has not been demonstrated in the hypobasal region, and further since the first appearance of such cells is at the base of the first leaf-sheath, the use of the term foot, with its dual significance, would seem to be inappropriate in the case of *E. arvense*.

AXIAL ORGANIZATION OF THE EMBRYO

In *E. arvense*, as has been shown, there is a very early delimitation of the apical cells of root and shoot, both being essentially aligned with the major axis of the archegonium (cf. Fig. 4A–C). This indicates that we have in this member of the Pteridophyta an embryo that is organized fundamentally in the same manner as that of the Bryophyta, i.e. one organized completely on a single axis. Since this is the only type of axis attained in the lower archegoniate plants—the bryophytes—it may be appropriately designated the primary or *bryophyte* axis. Its recognition should afford a valuable criterion in determining the phylogeny of organization in the higher archegoniates.

This use of the term primary axis differs from that of Hofmeister (1862) in that he considered the foot to be the entire primary axis. In applying his theory of axial organization to *Equisetum* he regarded the primary axis as abortive while the rapid development of the epibasal region led him to consider this as the secondary axis. His error in interpreting these facts lay in his failure to recognize that in this genus there is no secondary axis comparable to that found in other pteridophytes. Had he not been imbued with the concepts of leptosporangiate embryos he would probably have noted the fact that the embryo of *Equisetum* is organized on a single axis, of which his primary axis forms only the basal pole. It is interesting to note that since this keen observer regarded the foot as abortive he apparently saw no evidence of haustorial differentiation in the hypobasal region of *Equisetum*.

SEGMENTAL ORGANIZATION OF THE ADULT PLANT

The fact that many species of *Equisetum* will produce “adventitious” roots under various conditions is well established by the work of Hofmeister (1862), Goebel (1905) and Ludwigs (1911).

In our own study it was found that under greenhouse conditions immersing a shoot of *E. arvense* in water induced the production of roots in a very few days. The immersion of young secondary branches gave rise to buds that also developed into a shoot and a root, and buds were even obtained on tertiary shoots so formed. The latter material was found to be the most satisfactory for studying the early stages of bud development and was used throughout for this purpose.

There is disagreement as to the exact point of origin of these buds and details of their ontogeny are lacking. Hofmeister (1862) considers them to be formed endogenously, while Famintzen (1876), Janczewski (1876) and Campbell (1928*a*) state that they are developed exogenously. There is further disagreement as to whether or not they are formed in the axil of a leaf. Hofmeister (1862), Jeffrey (1899) and Bower (1908) consider that they are not axillary, while Campbell (1928*a*) states that they are always formed in the axil of a leaf.

In view of this lack of agreement a careful study was undertaken of the nodal region of tertiary branches, that had developed as indicated, in order to locate the position of the buds with respect to the leaves. Examination of the nodal region of tertiary branches so formed revealed the presence of an endogenous initial midway between two leaf bases. The apparent axillary position of such buds when seen in longitudinal section is due to the fact that the bases of the leaves are fused to form an enveloping sheath, so giving a false impression of the bud being axillary.

From a study of numerous sections it appears that the initial cell of the bud divides into two, forming an upper and a lower cell, and that these two cells follow closely the same course of division as in the proembryo—the shoot developing from the mass of tissue derived from the upper and the root from that of the lower cell (cf. Fig. 5). These two organs, therefore, as in the case of the embryo itself, are equivalent in origin and so are of equal status. The root in consequence can in no sense be regarded as adventitious to the shoot, a view generally held since Hofmeister's time. It is possible that the usual precocity in development of the shoot may have led to such a misinterpretation, but when the sequence of development is followed it is clear that the shoot and root arise as equivalent organs.

In regard to the bud that gives rise to the second segment of the sporophyte it was found that its initial was also endogenous, as both transverse and longitudinal sections of young embryos clearly show (Fig. 6A, B). There is also evidence to indicate that the development of the bud on the first segment follows the same course as that of buds in the aerial portion of the plant.

Thus from the facts presented it may be seen that the sporophyte of *E. arvense* has a very uniform and consistent mode of development. All buds are endogenous

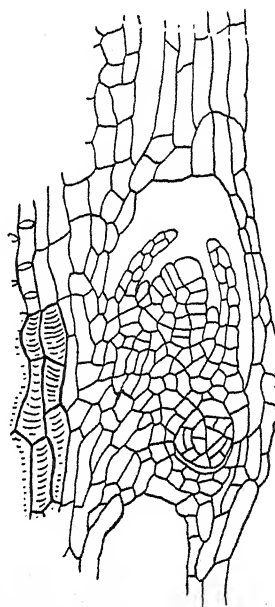


Fig. 5. Drawing of median longitudinal section of young aerial bud showing apical cells of shoot and root.

in origin and arise alternately with the leaf traces. Just as a dichotomy of the zygote gives rise to the first shoot and root as equivalent organs of the sporeling, so a bud on this first segment produces two similarly formed members, the same being true of all later buds whether both members develop or not, depending on environmental conditions. Thus, under normal conditions only the shoot member develops on the aerial portion of the plant, the corresponding root remaining latent. On the subterranean parts, on the other hand, the reverse is generally true—the root develops and the shoot is suppressed. That the latent potentialities can find expression, however, is clear from observations of plants under both natural and experimental conditions favourable to their development.

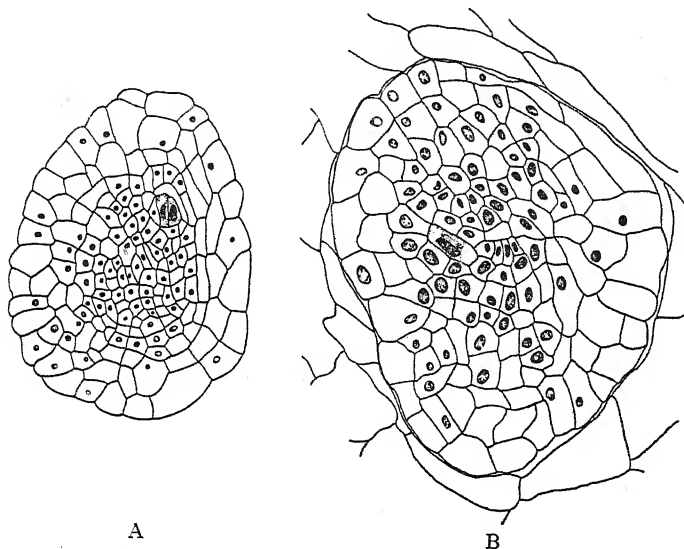


Fig. 6. Young embryos showing bud initial of second segment. A, transverse section (redrawn from Barratt, 1920); B, longitudinal section of a similar embryo.

SUMMARY

1. The cellular partitioning of the proembryo in *Equisetum arvense* and the delimitation of the apical cell of the root are held to conform to the theoretical laws enunciated by D'Arcy Thompson.
2. In the young embryo the absorptive process appears to take place over the entire surface. In later stages this function is assumed by the bases of the first-formed leaves.
3. Examination of early stages in the development of the embryo of *E. arvense* shows that it has its adult organs differentiated at the poles of the archegonial axis with the shoot at the apex and the root at the base—the primitive sporophyte axis.
4. All bud initials are endogenous in origin and alternate with the leaves, and parallel the developmental stages of the embryo.
5. The shoot and root are held to be equivalent members, either or both of which may be inhibited from development by unfavourable environmental conditions.

6. Because of the simple axial organization and the radial symmetry of its embryo and later stages, *E. arvense* is held to be one of the most primitive of the Pteridophyta, paralleling as it does the fundamental type of organization of the Bryophyta.

ACKNOWLEDGEMENTS

The writer is much indebted to Prof. R. B. Thomson for his advice and encouragement freely given throughout the preparation of this paper, and to Miss M. B. Givens for technical assistance in preparing certain of the sections examined.

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THE VALLEY FEN AT COTHILL, BERKSHIRE DATA FOR THE STUDY OF POST-GLACIAL HISTORY. II

BY A. R. CLAPHAM AND B. N. CLAPHAM

(With 3 figures in the text)

THE Cothill area, about six miles south-west of Oxford, is an irregularly shaped basin at the meeting point of several small streams. The water entering the basin by these streams, and also by seepage from its sides, is highly calcareous. The outflow stream, the Sandford Brook, runs into the Ock a little south-west of Abingdon. The area, which includes the Ruskin Reserve now administered by the National Trust, is one of several near Oxford noted for the richness of their fauna and flora and famous for centuries as collecting grounds. The reason for this is that these areas are too wet to be exploited except for litter and rough grazing so that their wild life has been little disturbed. After certain water-filled depressions at Cothill, colonized by *Phragmites*, *Juncus obtusiflorus* and *Schoenus nigricans*, had been interpreted as old peat-cuttings, it became a matter of interest to discover the thickness and age of the peat. Borings were made with a soil-sampling auger, and the stratigraphy and depth of peat were recorded along a number of transects both of the main and subsidiary valleys. The existing surface was levelled with a dumpy level and it was then possible to map the contours of the valley floor as well as the peat surface. Fig. 1 shows these floor contours, and also the boundaries (hedges, ditches, or streams) of the various enclosures into which the area is now divided. The present surface is much more uniform than the floor, showing a gentle steady slope down the various valleys towards the south-east corner of the area, where the Sandford Brook passes under the road to the disused mill.

The preliminary borings showed that the peat, most of which is highly calcareous, increases in thickness towards the south and east, attaining a maximum depth of 14 ft. in Morland's Meadow. The stratigraphy of a transect across this meadow was investigated more thoroughly with the help of a Hiller peat-borer, and a series of samples was taken from the deepest point for pollen analysis. The line of bores is shown in Fig. 1 A-C, and pollen samples were taken at B.

STRATIGRAPHY

Fig. 2 summarizes the stratigraphical findings along the transect of Morland's Meadow. The present surface slopes gently from north to south, with minor irregularities where small drains have been cut in an attempt to make the meadow suitable for rough grazing. Towards the southern margin a larger drain forms one edge of a narrow strip of carr whose southern edge is the main stream flowing from the Ruskin Reserve. Farther south the level rises, gently at first to String Lane, and

then rapidly up the side of the valley. It is evident from the diagram that this south side of the valley was formerly continued steeply to a point about 3 m. below the present surface, and then more gently to the lowest point 4.25 m. below the present surface. The north side rose fairly steeply at first, but then more gently. The floor of the valley is throughout a bluish green sand with a considerable fine fraction, and is

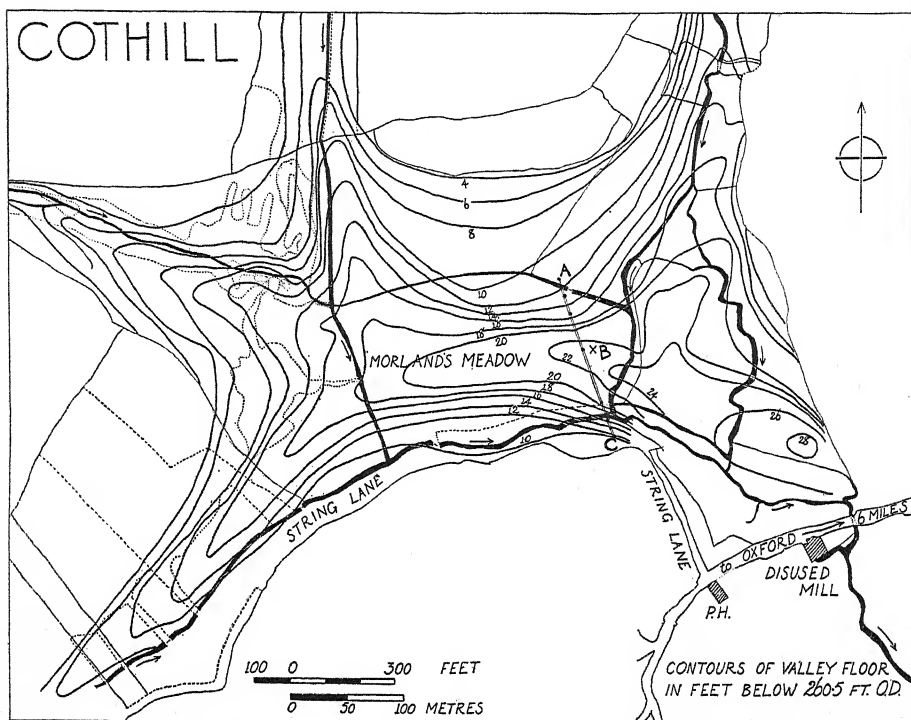


Fig. 1. Map of the valley-fen area at Cothill, Berks, six miles south-west of Oxford. The shape of the valley, as made out by boring, is indicated by contours at intervals of 2 ft. A-C is the transect shown in profile in Fig. 2. Samples for pollen analysis were taken from a boring at B. Streams are shown in black with the direction of flow indicated by arrows. The lightly dotted areas are peat cuttings.

evidently Calcareous Grit (Jurassic-Corallian). Occasionally fragments of rock were struck. These appeared to belong to deeper consolidated horizons of the Calcareous Grit.

The central borings show four readily distinguishable horizons. The top 15-25 cm. consists of a disturbed pale brown calcareous marl, with abundant tiny shells and with living roots. Below this is a thin layer of lake marl (Seekreide) consisting of almost pure calcium carbonate. There follows from 250 to 300 cm. of a heterogeneous and highly calcareous deposit with darker pockets and bands of lake peat in a greyish white granular and often shelly calcareous matrix (Kalkgyttja and Schneckengyttja). Plant preservation is poor, but recognizable remains include rhizomes of *Cladium*, monocotyledonous roots, seeds of *Menyanthes*, fruits of

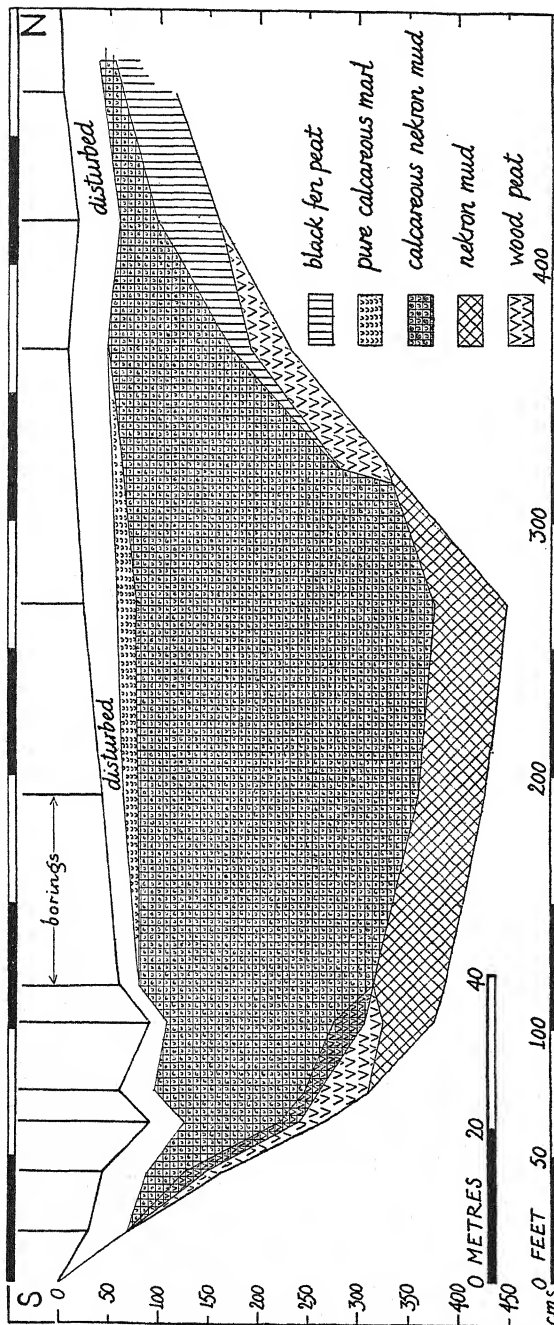


Fig. 2. Profile across the valley at Cothill along the line A-C of Fig. 1. The symbols employed are those of Faegri & Gams (*New Phytol.* 37, 329).

Cladium and *Carex* spp., and occasional small roots of *Corylus*. Finally there is a basal layer, 60–75 cm. thick, of a very homogeneous compact brown peat with no identifiable plant remains except *Carex* fruits and a few small twigs of *Salix*. This is evidently a lake peat (nekron mud, or gyttja) deposited in fairly deep water.

Borings nearer to the south margin of the valley differ in that there is no layer of pure calcareous marl, while woody layers, containing abundant nuts of *Corylus* and twigs of *Salix*, overlie the basal peat. *Corylus* roots are also found occasionally in this layer and in the calcareous peat above it. The basal peat becomes thinner and then disappears completely in borings close to both valley sides, woody peat with *Corylus* and *Salix* remains lying immediately over the floor.

This stratigraphical evidence points to an early phase of fairly deep open water, when the basal gyttja was deposited. Marginal woody vegetation of *Salix* and *Corylus* extended inwards as the water shallowed. This was perhaps a result partly of the accumulation of peat and partly of a lowered rainfall. Later followed the phase of formation of lake marl. The facts that the invading woody vegetation was destroyed and that the marl is highly calcareous, suggest an increased rainfall during this period, increasing the depth of water in the valley bottom, and raising the water table in the valley sides so that calcareous seepage water made a large contribution to the total inflow. During this period there must have been a marginal reedswamp with *Cladium* and *Menyanthes* as important constituents, and with occasional alder. Later, the layer of pure lake marl near the top suggests a further change in conditions.

PEAT ANALYSIS

Peat samples from a boring near the deepest point of the transect (*B*, fig. 1), were prepared for pollen analysis by the method described by Godwin (1939). Calcareous samples were treated initially with dilute hydrochloric acid and washed by centrifuging prior to alkaline maceration. Six slides were made from each sample, and counts were made along spaced traverses, using all the slides. Larger samples of peat were treated with dilute nitric acid and examined for plant remains other than pollen.

The results of the pollen counts and of the examination of other plant remains are shown in Fig. 3 and in Table I. The usual convention has been adopted of counting 150 grains of tree pollen (excluding *Salix* and *Corylus*), and of expressing relative amounts of the various kinds of pollen as percentages of this total. Numbers of *Salix* and *Corylus* pollen grains are then given as percentages of a total which excludes them. Notes were also made of the occurrence of other pollen grains and of fern spores.

Fig. 3 shows striking changes in the pollen content at different levels in the peat. An initial phase of *Betula* and *Salix*, with some *Pinus* (IV), is followed by a *Pinus* phase (V), then an *Ulmus-Quercus-Corylus* phase, with falling *Pinus* (VI), and finally, near the top, *Tilia* and *Alnus* increase rapidly (VII).

It has frequently been emphasized (cp. Godwin & Clifford, 1938) that the pollen rain falling on a peat surface is derived in part from trees growing on the peat or at

Table I

cm.	Non-tree pollen					Fern spores	Fruits and seeds	Wood	Rhizomes etc.
	Typha	Alsinoid	Caricoid	Eupatorium	Myriophyllum				
0									
20		+		+		+			
40						+	Menyanthes		
60						+			
80						+	Menyanthes		
100	+		Abt.			+			
120			+			+			Calliergon giganteum
140			+			+			
160			Abt.			Much			
180	+		+			+			Cladium
200	+		+			+			
220			+			Much	Carex		
240			+			+	Carex		
260						+			
280						+	Cladium		Cladium
300						Much		Charcoal	
320			+			Much			
340	Abt.	+	+		+	Much		Corylus (roots) Salix	Calliergon giganteum
360	Abt.	+		+	+	V. abt. (790%) V. abt. V. abt.			
380	+	+	+	+	+	+	Carex ? Cladium	Salix	
400			+			+			

its immediate margin, and in part from the general woody vegetation of the surrounding country. In extensive areas of peat formation such as the Fenland, the local component may be very important and may make it difficult to draw safe conclusions concerning the small distant component. For various reasons, however, it seems that the Cothill diagram really does reflect changes in the general woody vegetation and is not much disturbed by fluctuations in the local component. The basin is of very restricted extent, and there is stratigraphical evidence of its having had open water through most of the period of peat formation; *Corylus* pollen is very

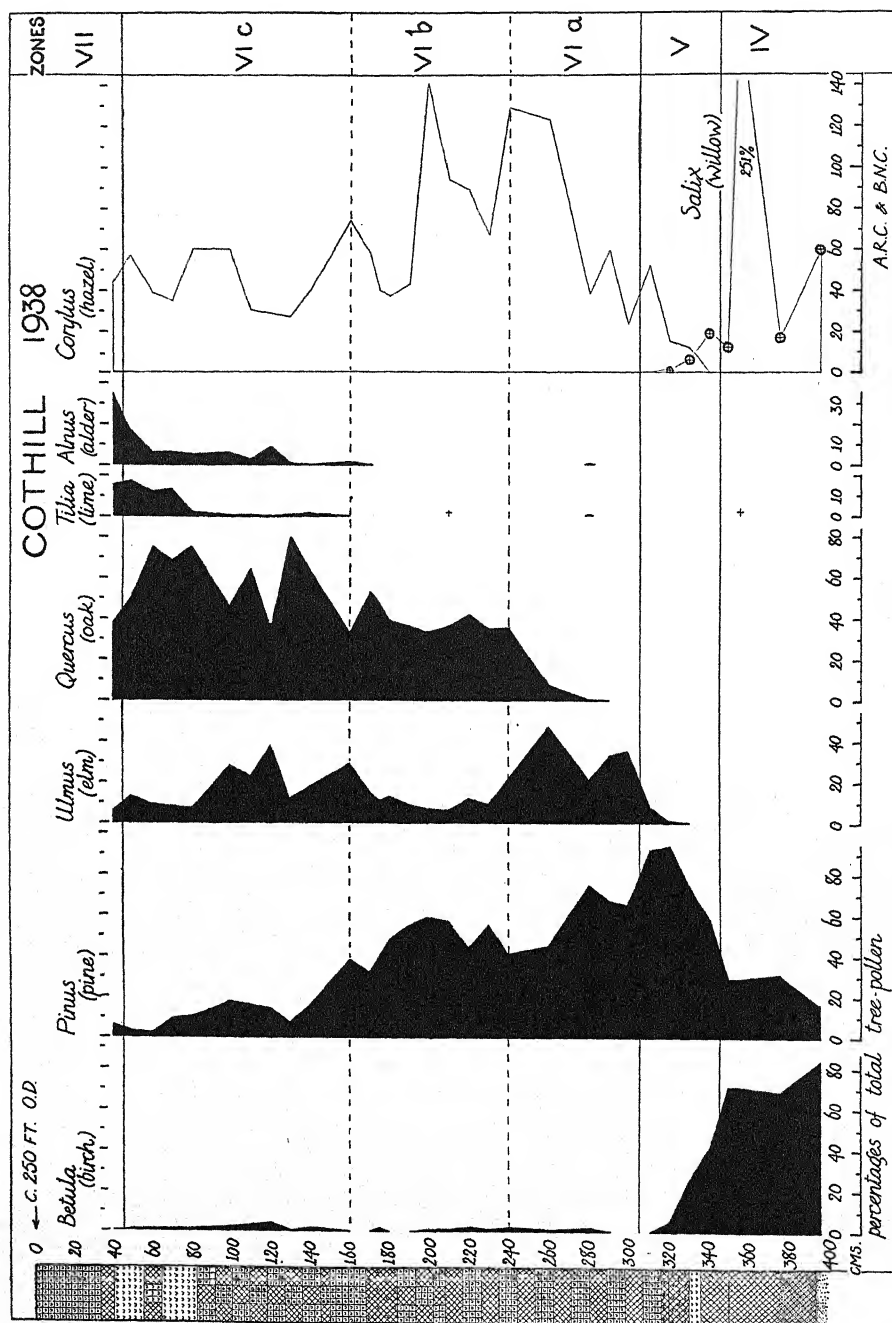


Fig. 3. Pollen diagram from site B of Fig. 1. Stratigraphical symbols as in Fig. 2.

scanty at levels where nuts and wood extend farthest inwards, becoming abundant much later; and, finally, the sequence of tree pollen maxima is identical with that found in other parts of England and generally in north-west Europe. The inference must be that through the period of peat formation there were changes in the woody vegetation of the surrounding country which left their record in the form of changes in the relative amounts of the various kinds of tree pollen preserved in the peat. These changes might be primarily the result of climatic changes, or of the successive immigration of woody species. That climatic change must be an important cause is suggested by the correlations with stratigraphical features (cp. Godwin & Mitchell, 1938; Godwin & Clifford, 1938). Local evidence against an interpretation exclusively in terms of successive immigration is provided by the appearance of occasional *Alnus* and *Tilia* pollen at much lower levels than those at which the pollen becomes abundant, suggesting that the trees were present but could not spread until conditions became favourable. Similar observations have been made at Tregaron, Cardigan-shire (Godwin & Mitchell, 1938), and elsewhere.

Comparison with other published diagrams makes it possible to identify the basal *Betula-Salix* phase with zone IV of Jessen (1935), and with zone D of Godwin & Mitchell (1938). This is approximately the Pre-Boreal climatic period. This identification is supported by the occurrence of a few pollen grains of *Myriophyllum alterniflorum*, a very characteristic plant of this period. During this period there must have been fairly deep water in the valley, with marginal reedswamp of *Typha*, *Carices* and perhaps *Cladium*, and with birch-willow-pine woods in the surrounding hills.

It seems useful to mark off the next higher zone (V) as that in which *Pinus* dominates the pollen diagram, with *Betula* falling rapidly, and with *Corylus* appearing for the first time. *Ulmus* enters in the upper half of this zone, but *Quercus* is very rare. Zone VI, characterized by falling *Pinus* and the prominence of *Ulmus*, *Quercus* and *Corylus*, occupies the greater part of the diagram. It can be divided into three fairly well-marked phases. There is first (VIa) a phase with high *Ulmus*, with *Quercus* absent or scanty, and with *Corylus* rising rapidly. The second phase (VIb) shows low *Ulmus* and *Quercus* almost as high as *Pinus*, while *Corylus* remains very high. In the third phase (VIc) *Pinus* is very low and *Quercus* high, *Corylus* is falling, and *Tilia* and *Alnus* appear consistently for the first time. Finally, at the top of the diagram, just below the disturbed surface layers, *Tilia* and *Alnus* increase rapidly at the expense of *Quercus* and *Ulmus*. This appears to be the well-known Boreal-Atlantic transition, so that our top zone (VII) corresponds with the beginning of the Atlantic climatic period. Then zone VI must correspond roughly with the Boreal period, zone V being transitional between Pre-Boreal and Boreal.

Reference to the stratigraphical diagram (Fig. 2) shows that the temporary inward spread of the marginal woody vegetation must have occurred in zone V, which may be regarded therefore as a relatively dry phase. There followed a rather wetter period, with destruction of this fringing carr and deposition of the calcareous nekron mud in fairly shallow water throughout zone VI. *Typha*, *Cladium* and *Menyanthes* must have been abundant in this period, probably in fringing reedswamp.

The sudden increase in *Alnus* and *Tilia* which marks the transition from VI to VII is characterized stratigraphically by the deposition of calcareous marl with few plant remains. We do not know the conditions under which such a deposit may be formed, but its restriction to the centre of the valley, and its coincidence with the rapid spread of *Alnus*, suggest strongly that the water-level was again raised at this period, presumably by an increased rainfall. It is in fact ordinarily supposed that the climate of the Atlantic period was warm and wet.

It is a striking fact that peat of earliest Atlantic age should extend almost to the present surface. More recently formed peat may have been destroyed during a subsequent dry period; or the present condition in which peat accumulation is prevented by a seasonal fall in water-level may have become established soon after the beginning of the Atlantic period. No evidence on this point is at present available.

ACKNOWLEDGEMENTS

We are very grateful to Mr H. Baker and Mr E. Arthurs for their assistance in the field; and to Dr H. Godwin, who gave us valuable help throughout, and especially in the zoning of the pollen diagram.

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NOTES

A DOUBLE NEEDLE FOR MOULD MANIPULATION

By G. H. GOSSOP¹

(With 1 figure in the text)

THE removal of small portions of moulds from Petri dish cultures, for mounting on microscope slides with the aid of a needle or forceps, is always a precarious operation, particularly in the case of floccose species, such as many of the *Penicillia*.

The double needle here illustrated has been found very satisfactory for this purpose. Its use makes it possible to detach fruiting bodies and other delicate structures with less risk of damaging them.

The device is easily made by binding together with thin copper wire two fine sewing needles, one about half an inch longer than the other. The points are placed level and the whole is secured by applying a little solder to the wire binding. The eye-end of the longer needle is fixed in the chuck of a watchmaker's needle-holder which is much better than the type of holder often supplied for laboratory use. It is very accurately made and will grip the finest needle.

The needle is best sterilized by dipping in spirit and burning off the surplus. There is then less danger of the solder melting due to overheating. Under these conditions the needles remain bright for a long period.

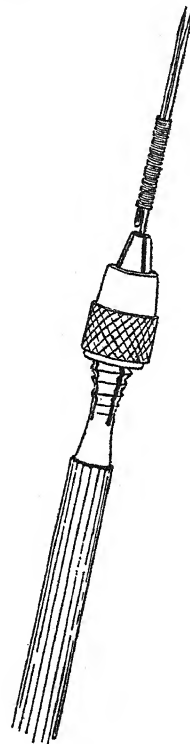


Fig. 1.

¹ Laboratories of Messrs John and E. Sturge (Citric) Ltd.

TECHNIQUE FOR THE OBSERVATION OF PROTOPLASMIC STREAMING IN SIEVE TUBES

By JAMES SMALL

Queen's University, Belfast

THE explanation of the very high rate of translocation of carbohydrates is one of the outstanding problems of plant physiology.

In a successful endeavour to observe streaming of protoplasm, or at least movement of particles, in mature sieve tubes the writer has devised a delicate technique with which it may be possible to make extended observations. Meanwhile he is of the opinion that, with the publication of the technique, the field of experimenters might be considerably widened.

The preparation of the tissue for observation is essentially a delicate operation. The elimination of the stoppage of streaming by shock, noted for other materials by Ewart (1903, pp. 72 sqq.), may require treatment as careful as that given to operations on the human eye. Acting upon this idea the writer had two ophthalmic lances, 2 mm. wide and 2 cm. long, specially made for these experiments by Messrs Grieshaber of Schaffhausen, and also obtained from the stock of the same makers two other ophthalmic lances, 3 mm. wide and 3 cm. long.

Young plants of vegetable marrow were transplanted into large flower-pots, one to each pot, and tied up to canes as they increased in length. A plant in its pot was transferred from the garden to the laboratory bench. A straight internode about 5 in. long was selected in such a position on the stem that this internode could lie across the stage of a microscope without any strain. Two transverse cuts were made about half-way through the stem with a razor blade, and a single longitudinal cut was then made with the smaller Grieshaber knife. The exposed part of the stem tissue was then placed against the lower glass of a Watson's live-box. The whole stem on either side of the raised part of the live-box was then padded to fit, and bound with two rounds of tape to the brass base of the live-box. The part of the stem being operated upon was thus firmly fixed, with tap-water and saliva keeping the cut surface moist.

On the opposite side of the stem two further transverse cuts were made with the larger Grieshaber knife, rather closer than the first pair, and with the same knife a longitudinal cut was made which left a thin slice connecting the two parts of the stem. Finally, with the smaller ophthalmic lance, a single slicing cut was made, which left parts of the connecting tissue thin enough to be viewed through the microscope (about three or four cells thick). The cut surfaces were kept wet with tap water and a brush throughout these stages. Then the thin connecting strip was flooded with tap water and a cover-slip placed on top. All the longitudinal cutting

had been done with the sharpest ophthalmic instruments known in Europe, on the principle that the sharper the cutting edge the smaller the shock during the operation.

Observations were made using a $\frac{1}{8}$ in. objective. Small granules in the sieve tubes were seen to be in fairly rapid movement. The movements were mainly smooth, but sometimes a larger granule would cease movement temporarily and begin again. The smaller granules appeared to move faster than the larger granules.

Naturally with this successful first experiment the writer appealed to other observers. A laboratory assistant was able to check one set of movements, as to reality and apparent direction, and went on to observe other movements also. Prof. D. C. Harrison of the Department of Biochemistry, Queen's University of Belfast, also checked the reality of the movements, so that there can be no doubt that movements have been observed in the mature sieve tubes of *Cucurbita Pepo* var. vegetable marrow.

The movements occurred in quite a number of sieve tubes, and were observed in both directions, up and down the stem. The speed of movement of some of the larger granules was timed with a micrometer scale and a stop-clock. The speed varied from 0.13 to perhaps 1.0 or more mm. per minute; 0.4 mm. per minute was the highest speed measured; more rapid movements were observed. The approximate speed in mm. per minute for other materials are: *Nitella* 2-3, *Vallisneria* 0.7, *Elodea* 0.96 (Ewart, 1903, p. 25). From the character of the movements it may be considered that the actual speed of the streaming may be higher than these first observations would imply, but this and other points, including the question of the observation of streaming in sieve tubes of other kinds of plants, involves careful development and extension of the technique which is here described.

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REVIEWS

A Laboratory Guide for a Course in General Botany. Fourth Edition. By L. BONAR, L. ROUSH and R. M. HOLMAN. Pp. 110 with 1 figure. New York: Wiley and Sons; London: Chapman and Hall. 1938. 6s.

Anyone who has organized laboratory classes in universities for some years must have acquired considerable wisdom of a technical kind concerning what it is practicable to ask students to do, and on methods of presenting material for study. This knowledge is rather seldom printed, and gets about surprisingly little. One is grateful therefore for this laboratory guide which expresses the practice of laboratory teaching in botany of the University of California.

The course resembles the first year introductory course in most English universities, with simple anatomy and morphology of the angiosperms preceding a review of the plant kingdom. It will be noticed that the physiology is of a distinctly elementary character, and would be improved by figures or descriptions of more exact experiments, such as one supposes are set up for class demonstration, of measurement of transpiration, stomatal opening, tropistic response and so forth.

There is a useful glossary and a long appendix full of tips for laboratory stewards and harassed demonstrators.

H. GODWIN

Encyclopédie Mycologique. IX. *Clés des Mucorinées (Mucorales).* By N. A. NAUMOV. Translated by S. BOUCHET and I. MOURAVIEV. Pp. 177 with 82 figs. Paris: Paul Lechevalier. 1939. Price 100 Fr.

The translation into French of the second Russian edition of Prof. Naumov's keys to the Mucorales, confers the benefits of this author's work upon a large number of readers. The aim of the author was to produce a workable key to the identification of species. This aim has been attained, with only such introductory remarks as are necessary for the full understanding of the keys. Although they are dichotomous, the usual failings of such keys are not present; the variable species and genera are not made to perch precariously on the ultimate branches, but may be found in several places at once, each form or variety resting comfortably in its place. This is especially noticeable in the keys to the large genera *Mucor* and *Rhizopus*.

It is claimed by the author that his descriptions are, except in a few cases, derived from monospore cultures. Moreover, the media were standard throughout, consisting of rice or potato; for it is pointed out that development on agar is often not characteristic. This suggests that the species and varieties here described are likely to be genetically sound.

However sound the descriptions may be, it comes as something of a shock that the number of species in the genus *Mucor* is 90, whereas the number in Zycha's monograph (1935) was 42. In view of such differences between the experience and judgment of Zycha and Naumov, the translators are to be congratulated on their foresight in including a comparison of the views of these two authorities in an appendix. The other appendices are most useful, especially the list of references to the original descriptions of species and genera. The appendix recording the observations on the sexuality of the Mucorales is both useful and interesting, but there seems less reason for its inclusion.

The book seems such a valuable addition to the *Encyclopédie Mycologique* that it may appear ungrateful to point out its shortcomings. Nevertheless, one cannot but notice certain obscurities in the keys. One example will suffice. The tribe Mucoreae is described on p. 12 as having "sporanges . . . toujours sans apophyse", whereas on p. 33, section XII, Absidioides of the genus *Mucor* is said to have "leur columelle . . . prolongée en une sorte d'apophyse". There is no description or figure in the text which would help to distinguish a true apophysis

from a "kind of apophysis". To continue with this example it may be seen on p. 77 that the striking similarity between Protoabsidia (*Absidia Blakesleana*) and the subsection Absidioides is noted, followed by the words: "Il est possible que l'étude du matériel vivant nous amène un jour à la nécessité de réunir tous les représentants de ces deux groupes dans le genre Protoabsidia." Is it possible then that the columella and its kind of apophysis of the section Absidioides have not been described from living material? The descriptions of the two species *Mucor pusillus* and *M. Hagemii* of this section are so detailed as to make this unlikely. Nevertheless one feels that it would have been valuable if those descriptions which are not original or which are not taken from living material had been marked in some way.

There is no doubt that the book will be extremely useful as a laboratory manual, and it seems a great pity that a volume of this kind should be bound in a paper cover. The back of the copy under review is already split.

J. L. HARLEY

Encyclopédie Mycologique. X. Le genre Mycena. By ROBERT KÜHNER. Pp. 710 with 239 figures and 16 plates. Paris: Paul Lechevalier. 1938. Price 300 Fr.

The book is a mine of information on many aspects of the study of Basidiomycetes in general, and of the genus *Mycena* in particular. The first part of the monograph is a general survey of the genus, in which the anatomy, ontogeny, cytology, and sexuality, are discussed to varying lengths. Here the argument is frequently supported by examples derived from the other genera of basidiomycetes, which not only increase the interest of the work, but help the reader to place in true perspective, the characteristics of the genus *Mycena*. The section on cytology and sexuality is extremely well constructed, and although at first one feels irritated by what appear to be detailed descriptions of well-known matters, one soon realises that it is the clarity of the argument which produces this illusion. Without doubt, this chapter stands out as an extremely able exposition of this controversial subject. Here the figures and diagrams are clear and useful. Elsewhere also the figures are usually good, but a few of them leave something to be desired, and some might have been more completely labelled.

The first chapter on methods includes details of technique for sectioning, fixing, staining, testing microchemically, and growing monospore cultures. The few lines dealing with the cutting of free-hand sections, may cause the reader to smile, and wonder if there is any necessity for their inclusion in a learned monograph of this sort. One must remember, though, that throughout the author is pleading for more attention from mycologists, to the microscopic characteristics of the fleshy fungi, and he is writing to a group of people who, he considers, have not yet fully realized the importance of these characters.

This attitude is also seen in the severity with which he treats the work of Murrill, who "a publié des diagnoses notoirement insuffisantes, n'indiquant jamais les caractères des cystides, . . . il est donc inexcusable et nous lui refuserons la priorité chaque fois que celle-ci sera en discussion."

While carefully weighing the value of data from various sources in a very unbiased manner, there are instances where the author is unable to accept evidence. Here he treats the work with quite straightforward sharpness which leaves no doubt as to his views. An example is seen on p. 99.

The general section ends with a review of past methods of dividing the genus, and finally a discussion of its affinities and limits.

This short chapter on affinities is a recapitulation of previous views, together with the author's own work on anatomy and the microchemical reactions of the hyphal walls. It is concluded, fairly convincingly, that there are no grounds for the retention of the genus *Omphalia*, which may be split into two, the one half being referred to the genus *Collybia*, and the other (the *Mycenarii* of Fries in the main), being absorbed into *Mycena* without difficulty. This conclusion is one which, it seems certain, will find more friends than enemies. The chapter ends with a warning against straining evidence in an attempt to reconstruct the phylogeny of the agarics, since the opinions expressed may remain for ever incapable of verification.

The next 510 pages are devoted to analytical keys to subsections and species. There are two keys to the subsections. The first is based on the author's system of classification, and here the characters used are those taxonomically most important, without reference to the ease with which they may be used. The second is for the purpose of more rapid identification of species, and uses first macroscopic characters followed by microscopic ones where necessary. Both keys are dichotomous and well arranged. The space given to each species is large, and the material presented includes original authorities, synonyms, full descriptions, and remarks, including habitats, variations and opinions. Often a figure is presented to elucidate some point. A few species, such as *M. rubromarginata*, are relatively little known to the author in the field. And for these, one, or usually more descriptions are obtained from observers knowing them well, coupled with further anatomical points derived from a study of preserved material.

The book ends with appendices of insufficiently described species, excluded species, and later diagnoses of new species, and, finally, bibliography and index.

One cannot help admiring the book, and wondering at the immense amount of knowledge that it contains. The general section makes excellent and profitable reading, and no doubt the keys will be found to be as workable as they appear.

J. L. HARLEY

An Ecological Glossary. By J. R. CARPENTER. viii+306 pp., with 7 tables and 5 maps. London: Kegan Paul, Trench, Trübner and Co., Ltd. 1939. 15s.

In order to review this list of terms which have been used by ecologists, twenty pages have been chosen at random and their contents analysed. These pages deal with 214 terms, so that there must be about 3060 in all. Approximately 52% are purely botanical, 13% zoological and 35% neutral or common to both branches. The predominance of botanical terms is partly due to the greater ease with which plant associations, etc., can be recognized, partly to the longer history of and greater number of workers in plant ecology, and, finally (in no small measure), to the work of Prof. F. E. Clements. Zoologists, especially as their work becomes more objective, find it very difficult to define animal communities, and until their ideas on the subject are less nebulous are not likely to need many technical terms. Even as it is, the number of zoological terms in the glossary has been swollen by including many which are not characteristically ecological and were not invented by workers who were primarily ecologists. Nevertheless, if the author's system were logically carried out, many more zoological terms should be included, such as density dependent factors, dulosis, haplometrosis, myrmecophile, pholophilie, phoresy, pleometrosis, symphile, synoekete, troglophile and troglonexene. I estimate that there must be at least a hundred and perhaps twice as many such terms omitted. Of the terms which are included, I consider that 13% should be omitted, either as being well known and without a meaning peculiar to ecology (hill, sea, etc.) or else scarcely ecological at all (reprint, separate, microcosm). The extent to which special terms are desirable is partly a matter of personal idiosyncrasy, but from the twenty sample pages I estimate that 64% of those included in the glossary are either synonyms or entirely useless. I believe that 36% is a generous estimate of the useful ones. It is symptomatic of this state of affairs that the more important a term is the more difficult it is to give an accurate and universally accepted definition. In my opinion very much more thought and much more objective field-work are necessary before an ecological glossary can fulfil much more than the rather secondary function of defining terms most of which need never have been invented. For this purpose the glossary, particularly if expanded and corrected, will prove useful. The numerous quoted definitions are valuable, but in many cases the author has not found it possible to trace terms to the work in which they were first proposed. It would need an expert botanist as well as a zoologist to criticize the glossary in detail. I have noted that the following terms are incorrectly defined: Dyar's rule, intrinsic factors, synechthran; the following are misspelt: acrodomatia, coprophagous, hedecaceous, pollenated, predromal, synechthren. I have the impression (perhaps due to my ignorance) that the botanical side of the work is much fuller and more accurate than the zoological.

O. W. RICHARDS

ON THE FACTORS AFFECTING THE MEAN SEED WEIGHT OF TOMATO FRUITS

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I. INTRODUCTION

THE weight of the seed in the tomato is to a large extent determined by the genetical constitution of the line. Lines homozygous for the factor **D** (tall, as opposed to dwarf habit) have relatively large seeds of mean weight between 3 and 4 mg., whilst lines homozygous for the corresponding recessive (**d**) have relatively small seeds whose mean weight lies usually between 2 and 3 mg. The wild species of *Lycopersicum*, which are closely related to the tomato, have smaller seeds than any of the cultivated varieties of *L. esculentum*, those of *L. pimpinellifolium*, for example, having a mean weight of only 1 mg.

Although determined primarily by the genetic constitution, the weight of the seed exhibits wide variation within the genotype. Random samples of seed collected in different years from the same pure line are frequently found to differ significantly from one another in their mean weight, as are also samples collected during the same season from different lots of plants of the same constitution. Much of this variation is no doubt due to differences in the external environment, since under normal conditions of cultivation no special precautions are taken to keep these external factors constant. More difficult to explain, however, are the variations in the mean weight of seed between fruits collected at the same time from a single plant, or from different plants of the same genotype, growing under identical conditions. As the figures in Table I show, these differences may be considerable, frequently being of the order of 60% of the mean seed weight.

Certain preliminary observations made in 1936 had indicated that these variations in seed weight within a pure line might be accounted for by variation in such factors as the number of fruits developing on the truss, the number of seeds developing in the fruit, and the position of the truss on the plant, but the data collected during that year were not extensive enough to allow any definite conclusions to be drawn. The observations were therefore repeated on a more extensive scale during 1937 and the results of this investigation are presented in this paper.

It will be observed from Table I that the variations in seed weight are accompanied by corresponding variations in the dry weight of the embryo, so that the ratio of mean seed weight to mean embryo weight for different fruits is practically constant. The weight of the embryo in the tomato is, in fact, very highly correlated

¹ A large part of the experimental work was done while the author was working in the Department of Botany at the University of Bristol.

with the weight of the seed (Fabergé, 1937; Luckwill, 1937, unpublished), so that the observations on variation in seed weight here recorded apply equally well to embryo weight. This fact is of considerable importance in view of the possible role of embryo size in the determination of the final size of the plant (Blackman, 1919; Ashby, 1937).

Table I. *Variations in seed weight and embryo weight between fruits collected on the same day from a single plant of line 105, homozygous for d, p, o, r, s, y, n (MacArthur, 1934). Each mean is based on twenty-four observations*

No. of fruits on truss	No. of seeds in fruit	Seed wt. mg.		Dry wt. of embryo, mg.		Ratio seed wt./embryo wt.
		Mean	S.E.	Mean	S.E.	
12	88	1.006	0.034	0.348	0.016	2.89
1	76	1.571	0.039	0.555	0.020	2.83
1	117	1.231	0.044	0.450	0.052	2.76
9	98	1.159	0.045	0.415	0.048	2.79
4	45	2.089	0.077	0.764	0.040	2.73

II. EXPERIMENTAL METHOD

The material employed in this research was derived from a commercial strain of tomato known as "Blaby", and was homozygous for the factors **D, P, O, R, Y, S, a**, (MacArthur, 1934). Altogether a dozen plants were grown. These were raised in pots in the greenhouse until about the middle of June, when they were transplanted, two to three feet apart, in a plot out-of-doors. Each plant was allowed to develop five laterals which were trained up bamboo canes, all other laterals being removed as soon as they appeared. The growth of these five laterals was stopped after the formation of the sixth truss of flowers. The number of fruits developing on each truss was controlled by removing all the remaining flowers and buds as soon as the requisite number of fruits had set. The number of fruits allowed to develop on a truss varied from one to eleven, and sufficient trusses were trimmed in this manner to provide about twenty fruits in each fruit number group, the distribution of trusses with varying numbers of fruits among the twelve experimental plants being entirely at random. The fruits began to ripen early in August and collecting was continued until the end of October, the number of fruits collected each month in the different fruit number groups being shown in Table II.

Table II. *Number of fruits collected*

Month	No. of fruits on truss											Total
	1	2	3	4	5	6	7	8	9	10	11	
Aug.	3	4	5	4	4	6	0	8	0	21	15	70
Sept.	10	12	9	12	11	11	0	0	1	0	7	73
Oct.	6	5	2	11	7	9	20	5	11	0	0	76
Total	19	21	16	27	22	26	20	13	12	21	22	219

Each fruit on gathering was given a serial number which was entered in a book, together with the corresponding plant number, truss number, number of fruits on the truss, approximate position of the truss on the plant (high or low), and the date of harvest. The fruits were picked as soon as they were ripe, and after weighing, the seeds were extracted from each fruit, enclosed in muslin bags and washed in running water to remove mucilage. They were then spread out on sheets of blotting paper to dry, after which they were stored in packets and were later counted and weighed, all the seeds in a single fruit being weighed together to the nearest 0.1 g. and the mean seed weight of a fruit being taken as total seed weight/seed number. Altogether data were collected in this manner from 219 fruits.

III. PRESENTATION OF DATA

(1) Preliminary analysis

The primary data are naturally far too extensive for publication so that only a table of means is given here. In Table III the mean seed weights, mean seed numbers, and mean fruit weights are classified according to (1) the number of fruits developing on the truss, and (2) the position of the truss on the plant, the lowest three trusses being designated "low" and the upper three trusses "high".

The mean seed weights recorded in Table III exhibit considerable variation, and in order to establish the significance or otherwise of this variation the seed weight data were subjected to statistical analysis. The analysis, which is presented in Table IV, is based on twelve observations in each fruit number group of which six came from a "high" truss and six from a "low" truss, so that altogether 132 observations were used in the preliminary analysis, giving 131 degrees of freedom. The "error" variance was computed from the sums of squares within the fruit-number-truss-position groups and the significance of the observed variances was determined by the application of Fisher's " χ " test (Fisher, 1928).

Table III. Mean fruit weights, seed weights, and seed numbers, classified according to the number of fruits on the truss and the position of the truss on the plant

No. of fruits on truss	Fruit weight (g.)		Seed no.		Seed weight (mg.)	
	Low	High	Low	High	Low	High
1	49.8	53.9	39.3	66.5	3.54	3.59
2	50.1	49.1	60.1	53.1	3.45	3.53
3	61.7	42.4	97.0	42.0	3.28	3.45
4	40.0	49.1	35.3	70.5	3.50	3.42
5	37.7	31.2	60.7	50.2	3.09	2.84
6	51.5	52.5	64.4	88.5	3.31	3.32
7	79.3	69.2	115.0	114.9	3.06	3.32
8	38.9	47.7	73.3	69.8	3.04	3.30
9	43.7	51.1	51.1	146.6	3.43	2.88
10	44.7	66.3	65.4	79.8	3.28	3.31
11	32.7	31.0	55.6	48.4	2.93	2.70
Means	48.1	49.4	65.2	75.5	3.27	3.24

Table IV. *Analysis of variance of mean seed weight*

Variance due to:	D.F.	Sums of squares	Variance	"z"	0.01 z
Position of truss	1	0.0001	0.0001	—	—
No. of fruits on truss	10	8.0697	0.8070	1.168	0.460
Interaction	10	1.2375	0.1237	0.240	0.460
Error	110	8.4300	0.0766	—	—
Total	131	17.7372	—	—	—

The analysis shows clearly that the position of the truss on the plant has no effect on the mean weight of the seed produced, there being no significant variance between the seed weight of fruits from "high" and "low" trusses. The variance in mean seed weight between fruits from trusses on which different numbers of fruits have been allowed to develop, however, is far too large to be accounted for by errors of random sampling and is therefore statistically significant.

(2) *Further analysis of data*

The preliminary analysis considered above has established the existence of significant differences in mean seed weight between fruits from trusses bearing different numbers of fruits, and it now remains to elucidate further the causes of these differences. This has been done by the technique of correlation and regression. Six correlation tables were constructed between the four variables in question, each table having 219 entries grouped into convenient classes. Correlation and regression coefficients between the six pairs of variables were calculated and their significance tested by the application of Fisher's "t" test (Fisher, 1928), with the exception of correlations involving fruit number as one of the variables, which could not be tested in this manner owing to the fact that this variable is not normally distributed, there being an approximately equal number of observations in each fruit number group (Table II). This objection, however, does not apply to regressions in which fruit number is the *independent* variable or to partial correlations in which fruit number is the eliminated variable. The coefficients of correlation and regression, together with the corresponding values of "t", are given in Table V.

It can be seen from Table V that the regression of mean seed weight on number of fruits per truss is nearly seven times its standard error and is therefore very highly significant. The corresponding regressions of fruit weight and seed number on number of fruits per truss are both less than twice their standard errors and are therefore of no statistical significance. The reduction in the number of fruits developing on a truss by the excision of some of the flowers and buds may therefore be expected to bring about an increase in the mean weight of the seed in the remaining fruits. The weight of the fruit itself, however, or the mean number of seeds per fruit, does not appear to be influenced by this treatment.

It can also be seen from Table V that there is a low but significant correlation between mean seed weight and the number of seeds in the fruit. The correlation

Table V. Correlation and regression coefficients between seed weight, fruit number, fruit weight and seed number in the tomato

Variables correlated		n	Correlation		Regression of <i>a</i> on <i>b</i>		0.01 <i>t</i>
<i>a</i>	<i>b</i>		<i>r</i>	<i>t</i>	<i>b</i>	<i>t</i>	
Seed wt.	Fruit no.	217	-0.4163	—	-0.0457	6.76	2.57
Fruit wt.	Fruit no.	217	-0.0846	—	-0.6334	1.25	2.57
Seed no.	Fruit no.	217	0.1628	—	1.6270	1.62	2.57
Seed wt.	Seed no.	217	-0.1867	2.80	-0.0014	2.80	2.57
Seed wt.	Fruit wt.	217	0.1754	2.58	0.0025	2.63	2.57
Fruit wt.	Seed no.	217	0.8057	20.03	0.4055	20.15	2.57

coefficient is negative, which means that high seed numbers tend to be associated with low seed weights. There is further a low positive correlation between fruit weight and seed weight which, however, is just significant on the 1% point of "*t*". Finally there is a very high positive correlation between the number of seeds developing in the fruit and the fruit weight. These correlations are discussed more fully in a later section of this paper.

(3) Partial correlations and regressions

The above analysis has shown that there are at least three factors (*viz.* fruit number, seed number, and fruit weight) which are significantly correlated with the mean seed weight in the tomato. The data in Table V, however, give little information as to the magnitude of the effects of these three factors on seed weight owing to the various interrelationships which exist between the factors themselves. In order to estimate the effect of each factor acting independently of the rest, the technique of partial correlation has been used. First order partial correlations were first calculated giving the correlations between successive pairs of variables with one of the remaining two variables eliminated, and from these partial correlations of the second order were calculated giving the true correlation between any two variables independent of variations in the remaining two variables. The general form of the equation used in the calculation of partial correlations of the first order was:

$$r_{12.3} = \frac{r_{12} - r_{13} r_{23}}{\sqrt{\{(1 - r_{13}^2)(1 - r_{23}^2)\}}},$$

where r_{12} , etc., represent the total correlation coefficients between various pairs of variables. The equation for the calculation of second order coefficients is similar, *viz.*

$$r_{12.34} = \frac{r_{12.4} - r_{13.4} r_{23.4}}{\sqrt{\{(1 - r_{13.4}^2)(1 - r_{23.4}^2)\}}}.$$

The results of these calculations are given in Table VI. Three second order correlations have been extracted, all of which are highly significant.

It is also possible from the sums of squares and products computed from the correlation tables to isolate the corresponding partial regression coefficients to satisfy an equation of the type

$$y = b_1 x_1 + b_2 x_2 + b_3 x_3,$$

Table VI. *Partial correlation and regression coefficients between the following variables*

1 = mean seed weight.
 2 = no. of fruits on truss.
 3 = mean fruit weight.
 4 = mean seed no.

Variables correlated		Variables eliminated	n	Correlation		Regression of a on b		0.01 t
a	b			r	t	b	t	
1	2	3	216	-0.4093	—	—	—	—
2	4	3	216	0.2876	—	—	—	—
1	4	3	216	-0.5624	10.00	—	—	2.57
1	2	4	216	-0.3982	—	—	—	—
1	3	4	216	0.5598	9.93	—	—	2.57
2	3	4	216	-0.1673	—	—	—	—
1	2	3 4	215	-0.3000	—	-0.0287	4.58	2.57
1	4	3 2	215	-0.5319	9.21	-0.0059	14.0	2.57
1	3	2 4	215	0.5450	8.14	0.0117	14.0	2.57

where x_1 , x_2 and x_3 represent the deviations from their respective means of fruit number, seed number, and fruit weight, y is the corresponding deviation of seed weight from its mean, and b_1 , b_2 and b_3 are the three partial regression coefficients. These coefficients have been calculated and are given in Table VI, together with the corresponding values of " t ". All are very highly significant.

IV. INTERPRETATION OF RESULTS

(1) *Seed weight and fruit weight*

The strongest of the three second order partial correlations is that between fruit weight and seed weight, which has a value of 0.5450 (Table VI). It seems clear that this correlation arises as a result of the common effect on both seed and fruit of fluctuations in various environmental factors during development. In the present experiment the plants, as far as was possible, were given uniform cultural treatment, but as the fruits were collected over a period of three months there was bound to be considerable variation in the conditions of temperature and water supply under which different fruits developed. The data show, however, that seed weight is less influenced by such variations than is fruit weight, which is probably due to the fact that the ovules develop within the fruits where the environment is relatively more constant than that in which the fruits themselves develop. The mean weight of all the fruits collected is about 50 g.; the mean seed weight is 3.25 mg. (Table II), so that for a change of fruit weight of 1 g. we should expect a change of seed weight of 0.065 mg. if both were affected proportionally. The actual regression of seed weight on fruit weight, however, is only 0.0117 mg. (Table VI), which is about one-sixth of this value.

(2) Seed weight and seed number

The negative partial correlation of 0.5319 between the number of seeds developing in the fruit and the mean seed weight is more difficult to interpret and the present research yields no information as to the causes of this relationship. It seems probable that it is a nutritional effect similar to that which gives rise to the negative correlation between birth weight and litter size in mammals (Kopéc, 1924), but it may possibly be dependent upon some spatial relationships in the young fruit, when the ovules are closely packed on the placenta. In this connexion it is interesting to note that Harris has found a similar, though lower, correlation between seed weight and the number of seeds developing in the pod in the bean (1914) and also in *Staphylea* and *Cladastris* (1911). The regression of seed weight on seed number in this experiment is -0.0059 mg. per seed, which, taking into account the large variations in seed number which occur in the tomato, is very considerable (see Table VIII).

Confirmation of this relationship deduced from partial correlations and regressions is given by the results of another experiment carried out in 1937. In this the data were all collected from a single plant and this was grown in a greenhouse so that environmental fluctuations were reduced to a minimum. Variation in seed weight due to differences in fruit number were also eliminated by allowing only a single fruit to develop on each truss. Altogether 7 fruits were collected from this plant, the seed number and mean seed weight of each was determined, and correlation and regression coefficients were calculated. The results are given in Table VII and it will be observed that the regression coefficient of -0.0066 agrees closely with the partial regression calculated from the main body of data.

Table VII. *Correlation between seed number and mean seed weight in subsidiary experiment*

Truss no.	1	2	3	4	5	6	7
Number of seeds	143	139	248	262	91	102	185
Total seed wt. mg.	457	454	737	684	358	385	588
Mean seed wt. mg.	3.2	3.3	2.9	2.6	3.9	3.8	3.2

Correlation between number of seeds and mean seed weight:

$$\left. \begin{array}{l} r = -0.8592; \quad t = 3.75 \\ b = -0.0066; \quad t = 3.90 \end{array} \right\} 0.02 \quad t = 3.36.$$

(3) Seed weight and number of fruits on truss

The coefficient of partial regression of seed weight on number of fruits per truss is -0.0287 mg. per fruit, which is somewhat lower than the total regression given in Table V, but is still highly significant. The correlation coefficient of -0.3000 also is somewhat lower than that first calculated and shows that the relationship between the two variables in question, although quite definite, is not a particularly close one. Like the correlation between seed weight and seed number just considered, it seems that this correlation must also be interpreted as an effect of nutrition, the removal of fruits from the truss resulting in an increased supply of food material to the remaining fruits and seeds. In this case, however, we might expect a negative

correlation to exist between fruit number and fruit weight as well as between fruit number and seed weight, but the fact that no such correlation was found does not necessarily invalidate this interpretation. It must be remembered that the water content of tomato fruits is high (60-70%), whereas that of the seeds is low (less than 5%), and for this reason it seems reasonable to suppose that a small increment in dry weight which would be readily detectable in the seed might pass unnoticed in the fruit itself or be masked by slight fluctuations in the water content.

(4) *The range of variation of seed weight*

This experiment has shown that the variation in seed weight (and embryo weight) within the genotype in the tomato may be classified under one of three headings as follows:

- (1) that due to variations in the external environment,
- (2) that due to variation in the number of fruits developing on the truss,
- (3) that due to variation in the number of seeds developing in the fruit,

and the partial regression coefficients given in Table VI enable the extent of the variation from each of these sources to be estimated. As has been pointed out on p. 186, any variations in the external environment in which the fruits developed will affect both the seed weight and the fruit weight, so that the partial regression of seed weight on fruit weight may be taken as a measure of the effect of such fluctuations on the mean seed weight of the fruit. The regression coefficient is 0.0117 mg. per g. and the maximum range of variation in fruit weight within any one seed number group (since seed number and fruit weight are correlated) in this experiment is about 60 g. This means that the maximum possible variation in seed weight due to inequalities in the external environment is $60 \times 0.0117 = 0.702$ mg., which is about 20% of the mean weight of all the seeds collected. Similarly, the number of seeds in a fruit varies from 1 to 250 and the regression of seed weight on seed number is -0.0059 mg. per seed, so that the maximum variation in seed weight from this source is 1.475 mg. or 45% of the mean seed weight. A similar calculation for fruit number shows that a reduction in the number of fruits developing on a truss from 11 to 1 may be expected to bring about an increase in the mean seed weight of approximately 10%. As the figures in Table VIII show, the maximum possible variation in mean seed weight from all three sources is of the order of 75%. The maximum range of variation recorded in this experiment was about 55%, which is well within the calculated limit.

Table VIII. *Factors correlated with the mean seed weight in the tomato*

Correlated variable	Range of variation	Partial regression coefficient	Max. range variation in seed weight (mg.)	Max. range variation as % mean
No. of fruits on truss	1- 12	-0.0287	0.316	10
No. of seeds in fruit	1-250	-0.0059	1.475	45
Fruit weight	60	0.0117	0.702	20
Total maximum variation =			2.493	75

Range of mean seed weight recorded in this experiment = 2.3 mg. to 4.1 mg. = 1.8 mg. = 55% of the mean seed weight.

VI. SUMMARY

It is found that significant variations exist in the mean weight of the seed collected from different fruits of the same genotype in the tomato.

These variations in mean seed weight are accompanied by corresponding variations in the mean dry weight of the embryo.

The position of the truss on the plant is found to have no effect on the mean weight of the seed.

It is established by the technique of correlation and regression that these variations in seed and embryo weight are attributable to three causes, viz.:

- (i) variation in the external environment,
- (ii) variation in the number of fruits developing on the truss,
- (iii) variation in the number of seeds developing in the fruit.

The maximum amount of variation attributable to each of these three sources is estimated from the partial regression coefficients.

I wish to record my sincerest thanks to Mr E. J. Hatcher of Bristol for his invaluable assistance in collecting the data for this analysis, without which I should have been unable to complete the experiment.

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CORTICAL AIR SPACES IN THE ROOTS OF *ZEA MAYS* L.

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(With Plates I and II)

SACHS (1882) placed on record what seems to be the first critical study of the structures with which this investigation is concerned. He divided the large air-filled lacunae found in many plants into two groups. One he held to be due to "the splitting of the partition wall (between cells) and the growth of its now separated lamellae", while the other arose from "the cessation of growth of inner masses of tissue and their drying and splitting, while the surrounding tissues continue to grow". De Bary (1884) applied the adjectives schizogenetic and lysi-genetic to the two types, the latter of which forms the basis of the present paper.

References to air spaces and their probable value for the aeration of tissues in water plants are scattered through the literature, but comparatively few attempts have been made to extend our knowledge of their development beyond what is contained in Sachs's statement. Interest has not been entirely lacking, however, and a search of the literature disclosed eight papers touching on this phase of the subject.

Norris (1913) carried out a number of experiments, growing *Zea Mays* in various soils and water cultures. He found that the air spaces were invariably present in water cultures, but were reduced in roots growing in porous soils, and from this concluded that "the development of air spaces appears to depend largely upon the quantity of air available in the medium surrounding the root".

Hunter (1915), after investigating the condition in *Vicia Faba*, suggested that the boring action of the lateral roots may be responsible for some of the extensive system of air spaces found.

Folsom (1918) reported that in roots of *Ranunculus sceleratus* the amount of aerenchyma varied directly with the water supply except in the case of totally submerged plants. In *R. abortivus* roots it varied inversely with the water supply except when the plants grew under pronouncedly xerophytic conditions.

Miss Snow (1920), working with *Scirpus validus*, was unable to influence the production of air spaces consistently by variations in atmospheric pressure.

Miss Dunn (1921), working on Marquis wheat and White Dent corn grown in solution cultures, found the cortical lacunae invariably appearing in the roots. They were not observed in sand or soil cultures of wheat, either in summer or winter, but none of the cultures was continued for more than thirteen days. In the Indian corn, she invariably obtained cortical lacunae, and noted that the time of appearance seemed to vary with the rate of growth of the roots.

Stover (1928) published a research on the roots of *Zizania aquatica*. He

attributed the cortical air spaces to the pulling apart of cells and the final destruction of some, by the enlargement of a ring of mechanical tissue in the outer cortex.

Severin (1932) gave a similar account of the development of lacunae in *Sagittaria* roots.

Bryant (1934) found that the cortex of roots of barley grown in aerated culture consisted of uniformly compact parenchyma with no conspicuous intercellular spaces. In non-aerated solutions, this region was composed of large air spaces separated by narrow strands of parenchyma.

Since, in the present investigation, the chemical composition of the cell wall was found of importance, a brief consideration of the literature on this subject is necessary.

Mangin (1888, 1889, 1890*a*, 1890*b*, 1891, 1893) was the first to study critically the chemical constitution of the middle lamella in plant tissues and concluded that it was pectic in nature. He made use of ruthenium red for the detection of pectic substances. Later, Allen (1901) corroborated his conclusions.

Support was given to this conception of the middle lamella by Molisch (1923) who, however, qualified his view with the statement "es ist nicht unwahrscheinlich, dass die Mittellamelle nicht immer dieselbe Zusammensetzung hat".

On the other hand, Tupper-Carey & Priestley (1923) endeavoured to show that the middle lamella in the meristems of root and shoot was not merely pectic acid or its salts, but rather composed of a protein-pectin complex. In the adult parenchyma, however, they stated that the middle lamella was composed of calcium pectate and calcium soap.

Cormack (1935), in a research on the formation of root hairs, stated that the middle lamella, at first of soft pectic acid, changes to hard calcium pectate under proper conditions, namely, a pH in the neighbourhood of 6.0 in the presence of available calcium.

The secondary wall of cells such as those with which we are concerned is generally agreed to be cellulose.

In the present investigation an attempt has been made to throw some additional light on the development of air spaces and the causal agencies concerned. It deals in particular with cortical spaces in the roots of certain members of the Gramineae. For the most part Indian corn of the variety Yellow Dent was used, having been selected as an easily available, rapidly developing form and one known to form large cortical air spaces. As the investigation proceeded, however, comparative work was done on some other species. The anatomy, microchemistry and experimental ecology of lacuna formation were the three main aspects considered.

For the anatomical study of air spaces, seedlings of *Zea Mays* were grown seven days in tap water, fixed in chromic-acetic-formalin, and dehydrated, using a series of alcohols 2.5-100% with eleven intermediate concentrations. The dehydrating had to be carefully done to avoid collapse and distortion of the tissues. The pieces of root were lightly stained *in toto* in Mayer's carmine as an aid in orienting the material, then cut at 5 μ for tip sections and 10 μ farther back. The microtomed sections were then restained in Ehrlich's haematoxylin.

ANATOMY AND MICROCHEMISTRY

Pl. I, fig. 1 shows a transverse section of a typical root at 115μ from the root apex. In the outer portion is a ring of root cap tissue, two to three cells in thickness, which surrounds the section. Inside this is to be seen a single row of radially elongated epidermal cells. In the centre of the section is the stele, a circular area composed mostly of small, compact cells, but showing four large thin-walled cells which resemble vessels. Between the stele and the epidermis is the cortex, composed of cells which tend to be tangentially elongated. Through a pulling apart of these cells at the corners there arise small diamond-shaped or triangular intercellular spaces shown at a higher magnification in Pl. II, fig. 1. These spaces were first noted at 40μ from the root apex. As the cells gradually enlarge by vacuolating, they become more oval in cross section and the spaces increase in size. A few rows of cells adjoining the epidermis tend to remain small, dividing more rapidly than the inner ones. The large cells divide in either tangential or radial plane, apparently without regard to the direction of their major diameter.

In the first half millimetre from the root apex there occurs the development of relatively small schizogenetic spaces. These are largely blind, being more or less closed off above by other cells. The cells continue to enlarge and the spaces at the corners gradually increase in size.

Pl. II, fig. 2 is of a transverse section 505μ from the growing point. Here, in the central part of the section, is seen, for the first time, a cell separated from its neighbours on both its radial faces. This cell has clearly failed to keep pace with its neighbours in rapidity of vacuolation. No evidence of complete collapse of cells was seen at this level, however, and the protoplasm of all the cells is still fairly dense.

So far nothing has occurred that might not be found in a normal root. In the stage illustrated in Pl. II, fig. 3, however, the development has gone farther. This part of the root is still elongating somewhat, but even the most backward cells have almost completed their vacuolation. They have swollen and pulled apart until flattened areas of contact are comparatively rare and of small extent. Moreover, there is a significant difference in appearance between the cell contents shown in this figure and in Pl. II, fig. 2. In the section illustrated in Pl. II, fig. 3 either a considerable part of the protoplasm has disappeared, or it has become changed in such a way as to remain unstained by the haematoxylin. Moreover, some of the cells have apparently lost their ability to maintain turgor pressure, the walls adjacent to the air spaces having become concave rather than convex as in Pl. II, fig. 2. In the centre of Pl. II, fig. 3 is an irregular empty space as large as two or three cells. This is also a schizogenetic space. No fragments of empty cell walls are to be seen, but three of the cells surrounding it, two to the upper right of the space and one to the lower left, have decreased in size sufficiently to leave the large space.

Pl. II, fig. 4 is from a region somewhat higher in the root than Pl. II, fig. 3. No large space is visible in this section, the cells still remaining in contact. The collapse of the cells in general has proceeded farther, however. In many cases the walls, where not adhering to other cells, have been drawn in until the cell in section has taken the shape of a cross or an irregular several-armed figure.

This is exactly the appearance figured and described by Stover (1928) for *Zizania aquatica*, and by Severin (1932) for *Sagittaria latifolia*. Both these authors consider the appearance to be due to a stretching of the cells by the growth of hypodermal tissue of the root. A comparison of Pl. II, figs. 3 and 4, both at the same magnification, will show the fallacy of this assumption and provide a proper conception of what is taking place. A comparison of the size of cells in the two figures shows that the arms are not drawn out from the cells by growth of the surrounding tissues, but are produced by shrinkage of the cells themselves.

As one proceeds farther from the root tip, spaces like that shown in Pl. II, fig. 3 increase sporadically in size and number, though for some time they are scattered, and transverse sections devoid of them may be obtained. Pl. II, fig. 5 is of a space that has developed farther than that in Pl. II, fig. 3. Some of the cells have lost all their protoplasm and appear only as collapsed walls extending into the space.

In extreme cases the breakdown of cells proceeds until, at some centimetres from the tip, the appearance shown in Pl. I, fig. 2 is presented. In this section the protoplasm has disappeared from all the cells of the cortex with the exception of the outer mechanical tissue and a few strands of living cells extending from it to the endodermis. This section is from a root grown in a liquid medium. In roots growing in well aerated soil, the breakdown does not go to such an extreme, and a considerable portion of the cortex remains with air spaces scattered through it.

From the work so far described, it is clear that the formation of spaces is accompanied by the death of the cells. The question remains whether either of these phenomena may be supposed to have a causal relation to the other. Stover (1928), in his paper on *Zizania* says: "The continuation of the enlargement of the cortex tears these cells apart completely, or actually destroys some of these cells, until the mature cortex shows radiating plates of cells with the larger cavities bordered with the torn fragments of cells." It seems clear that his view is that the tearing apart and also the death of the cells is due to the growth of other cells and that destruction is merely a by-product of air space formation.

Since the observations so far described seemed to cast doubt on this theory, further studies of the death of the cells were made. The use of vital stains has been recommended for this purpose. Neutral red, congo red, methylene blue and Bismarck brown were used on rather thick transverse and longitudinal sections. Only neutral red gave really satisfactory results, methylene blue being the next best. A modification of the method of Luyet (1937) was followed, using neutral red as the vital stain. Luyet reports that dead cells in the epidermis of *Allium cepa* took an intense orange colour, whereas the living cells became a cerise red colour. In the cortical cells of corn roots the living cells took on a bright red hue, whereas the dead cells were practically colourless.

The actual staining was carried out in the following manner. The sections were put in a 0.5% solution of neutral red to which had been added a trace of dilute potassium hydroxide solution which gave the stain a light orange-red colour. The sections were left in the solution five minutes or more. They were then washed in tap water and mounted in distilled water. The contrast between dead or injured

(in sectioning) and living cells was extremely clear. The living cells stained bright red but the dead cells failed to stain appreciably, with the exception of granules and, in some cases, the collapsed cytoplasmic membrane. The onset of death in many stained cells could be detected by the fact that the nucleus, which normally remained colourless, became deep red in colour. Since a large number of cells was injured in transverse sections, most of the work was confined to longitudinal sections.

Examination of the stained living cells revealed that in many cases streaming could be seen in the cytoplasm, but this could never be found in unstained cells. In the case of the stained cells, plasmolysis took place readily when they were placed in a strong (40%) sucrose solution, whereas the unstained cells failed to plasmolyse. Using the three criteria of vital staining, streaming and plasmolysis, it was easy to distinguish the living from the dead cells.

In the region where air-space formation was taking place but where the spaces had not reached their maximum size, dead cells were numerous. Such spaces were usually surrounded by collapsed dead cells, with an occasional living one bulging into the space due to its turgor pressure. No evidence of abnormal stretching was to be found in any of the cells, whether dead or living.

Other reasons for this opinion also became evident as the work proceeded. As will be recounted later, a method was devised by which roots of Indian corn entirely free from air spaces could be produced. Investigation of such roots using vital staining revealed that a very much higher proportion of the cortical cells stained than was the case in corresponding sections from roots in which air spaces were being produced. Also, roots were allowed to grow until they reached a length of thirteen to fifteen centimetres under conditions which caused no air space production, so that there were present several centimetres of mature cortex which had reached its maximum diameter. Then half were kept under the same conditions and half put under conditions which assist air-space formation. It was found that in the former the cells were all turgid, of normal shape, and no spaces were present. In the latter, death of the cells and extensive normal space formation ensued both in the mature tissue, which was formed before transference, and in the newly formed cortical tissue.

The weight of evidence is for formation of these spaces as a direct result of the deterioration and eventual death and disappearance of the protoplasm, with accompanying collapse of the cell walls.

In the material so far considered any of the cortical cells, with the exception of a few rows just under the epidermis, may increase in size, then die and collapse, leading to the formation of spaces. Sometimes, as in Pl. I, fig. 2, almost all the cells do meet this fate. In other cases a larger proportion remains alive. In no case, however, does a cell in this region die without its walls collapsing to increase the size of lacunae.

An investigation of the composition of the cell wall threw light on the reason for this invariable collapse. Tests for cellulose in the secondary walls were made with iodine and sulphuric acid, chloriodide of zinc, and copper oxide ammonia. Roots

grown in tap water and between damp cloths were used and series of sections were cut from the tip backward.

Tip sections were treated with dilute iodine potassium iodide followed by 75% sulphuric acid. The result was a brownish-yellow colour in the walls owing to iodine colouring the protein material in them. After treatment with hot 2% potassium hydroxide for twenty minutes, the walls still gave no cellulose reaction. However, after a previous treatment with hot Eau de Javelle for ten minutes a dark blue colour resulted. At one centimetre from the tip, the sections stained a deep brown colour in the walls with a lighter brown in the protoplasm of both cortex and stele. After a twenty-minute treatment with hot 2% potassium hydroxide, such sections still showed the deep brown colour in the cell walls on treatment with iodine potassium iodide and sulphuric acid, but a bluish tinge could be detected here and there. At four centimetres from the tip, sections untreated with potassium hydroxide had cell walls stained brown with tinges of blue. After a previous treatment with hot 2% potassium hydroxide, similar sections showed root hairs blue, epidermis dark blue, hypodermis yellow, cortex dark blue, endodermis yellow and stele dark blue. The colour was permanent. There was considerable difficulty in obtaining penetration in untreated sections in this test for cellulose.

With chloriodide of zinc, tip sections remained yellow. Those at one centimetre from the tip turned blue in the cortex, except for the endodermis, and a ring of narrow cells two to three rows thick just within the epidermis which remained bright yellow. The stele was also blue. All sections above this level gave similar results. The partially collapsed cells surrounding air spaces stained more quickly and a deeper blue than cells still turgid.

It is clear that cellulose is present in the walls even of very young cells, but its presence is masked by the protein which permeates their walls. The masking effect decreases as the cells become older, disappearing more rapidly in those that are to collapse and form air spaces.

The middle lamella in the walls of all the broad cortical cells stained bright red with ruthenium red, indicating pectic material. The exact nature of this material was indicated by differential solubility methods. It failed to dissolve when heated to 85° C. for 1 hr. in distilled water, which indicated that it was not pectin. A similar treatment for one half hour with 2% potassium hydroxide solution caused the cells to separate, and removed the pectic material, indicating that it was pectic acid rather than an insoluble pectate, which would have necessitated previous treatment with dilute acid to bring about solution.

The fact that these walls contained no other cell-wall material except cellulose and pectic acid was shown by their complete disappearance when the potassium hydroxide treatment followed one in which copper oxide ammonia had dissolved the cellulose.

Tests were made to determine the nature of the walls of the outer ring of cells which failed to stain blue with chloriodide of zinc and which never collapsed to form spaces. Sections were put in yellow methyl red solution and the middle lamella of these cells turned red, with a tinge of pink in the secondary walls. In phloroglucin

and hydrochloric acid the middle lamella turned violet-red at once. When treated successively with copper oxide ammonia to dissolve cellulose and hot 2% potassium hydroxide solution for pectic acid, these walls were still visible, but when such treatment was preceded by soaking in hydrogen peroxide no vestige of wall was left. The evidence is thus clear that the walls of these cells, particularly the primary walls, are lignified. The necessity of using both copper oxide ammonia and potassium hydroxide indicates also the presence of cellulose and pectic acid.

EXPERIMENTAL ECOLOGY

The purpose of the experimental work was to separate the factors which determine air-space formation from those which do not. In order to do this, an exhaustive series of tests was carried out concerned with each of the factors which it was felt might possibly have a bearing on the question of accelerating or inhibiting lacuna formation.

As a preliminary the question of the normality of lacuna formation in the roots of *Zea Mays* arose. To settle this question, preserved material of the roots of *Z. Mays*, grown for half a season in a field, was examined. Air spaces were quite prominent and numerous in the root cortex. Therefore, air-space formation does take place under the normal conditions affecting plant growth in the open.

For use in the experiments Indian corn grains were allowed to germinate between damp cloths or blotters until the radicles were $1-1\frac{1}{2}$ cm. long. This was made the standard length for the experiments, which were largely carried out in water cultures over periods four days in length. The radicles of six seedlings were put through holes in a flat cork float, $\frac{1}{4}$ in. thick, which rested on the surface of the solution. Amerseal jars were used and capped to prevent the grains from drying out. Where aeration, oxygenation or stirring was carried out, the lids had the necessary holes punched in them to admit the glass tubing or stirrer.

The first experiments were planned to ascertain the influence of water supply. From the beginning it was evident that the presence or absence of excess water around the roots exerts an influence on air-space formation. Relatively dry soils lead to smaller and fewer spaces in corn roots than water laden soils. Corn seedlings grown in various water cultures in the usual laboratory manner inevitably have large air channels. Moreover, experiments conducted with seedlings whose roots grew down into water-saturated atmospheres showed that merely surrounding the grain with cotton batting soaked in distilled or tap water aided space formation. Air spaces were large after three days in such an experiment at 20° C. If, on the other hand, the grains were surrounded by filter paper made barely moist by distilled or tap water, the formation of air spaces was retarded. In a typical experiment of this kind, even at the higher temperature of 22° C., there were no spaces in the majority of roots after 4 days (one root had a few tiny spaces). These results suggest the question as to whether the abundance of water is the direct cause of increased space formation or produces the lacunae indirectly by the exclusion of some other substance such as oxygen.

A series of experiments to settle this question was devised. Corn seedlings were grown in tap water and Rothamsted¹ culture medium. These were kept briskly agitated by mechanical stirrers so as to provide a more concentrated solution of oxygen from the atmosphere and to prevent a stagnation of unaerated water about the roots. This was ineffective. The spaces were as large as in check experiments where the liquids were unstirred. A more complete method of aeration was accomplished by passing a strong current of compressed air into the water through a Berkefeld filter. In this way the water was kept thoroughly stirred and was filled with small air bubbles throughout the experiment. The result of this treatment was that the size of the air spaces was reduced and this result was consistent through a series of temperatures from 20 to 35° C. Air spaces were never completely eliminated, however.

The logical sequel to this was the use of still higher concentrations of oxygen. For this purpose a cylinder of commercial oxygen was used. Oxygen was passed through an easily regulated pressure gauge and a capillary tube into the solution. The end of the capillary tube was pressed against the bottom of the jar to split the outgoing oxygen into fine bubbles. Slow oxygenation proved to be inefficient, but reduced the spaces somewhat. It was found necessary to oxygenate the solutions for several hours before putting in the seedlings in order to get consistent results. Rapid passage of oxygen through the culture or somewhat slower oxygenation supplemented by a motor driven stirrer resulted in the complete elimination of air spaces. It is apparent from this that the cortical cells require that a high concentration of oxygen be continually maintained in the medium surrounding the root if they are to remain in position and not collapse. From the experiments it also became evident that aeration or even slow oxygenation without stirring did not accomplish this, but that, on the other hand, rapid oxygenation or slower oxygenation supplemented by a motor driven stirrer could renew the oxygen supply around the root quickly enough to prevent a deficit.

While aeration was found to be beneficial to the growth of roots, heavy oxygenation tended to retard growth in length. Since Miss Dunn (1921) has stated that "the time of appearance of the cortical openings in sand or soil cultures of White Dent corn seems to vary with the rate of growth of the roots", the question arose whether a mixture of oxygen and nitrogen could be found which would prevent lacuna formation while still permitting rapid growth. In experiments to solve this problem Rothamsted culture solution was used, since it allows normally rapid growth without appreciably affecting air-space formation. The experiments showed that an equal mixture of oxygen and nitrogen bubbled vigorously though the solution would practically inhibit the formation of lacunae for the four days that the experiments lasted and at the same time would permit excellent growth of the root. In fact, at a high temperature (35° C.), growth in this concentration of oxygen was more rapid

¹ Potassium nitrate 1.0 g., magnesium sulphate 0.5 g., calcium sulphate 0.5 g., sodium chloride 0.5 g., potassium orthophosphate di-H 0.3 g., potassium orthophosphate mon-H 0.27 g., ferric chloride 0.04 g., water 1 l., pH 6.2.

For pH 3.8 use potassium orthophosphate di-H 0.5 g. instead of mixture.

than when the solution was kept saturated with air or pure oxygen, and, while a few cells in a small percentage of the roots might have collapsed, typical lacunae were entirely absent. At this temperature the length of roots after four days using air was 6.5–11.0 cm. With 50% oxygen plus 50% nitrogen it was 9.0–14.0 cm., and with a strong stream of pure oxygen 4.3–8.0 cm. At 20° C. the lengths with the same three degrees of oxygenation were 12.2–13, 10.0–13.3, and 3.8–5.2 cm. respectively. In this case saturation with an equal mixture of oxygen and nitrogen once more eliminated air spaces, but optimum growth was not obtained. The pure oxygen also appeared to exert a greater effect in retarding increase in length than at 35° C.

Confirmatory results were obtained when roots were allowed to grow in gases rather than liquid media. In moist air at room temperature, air-space formation could not be eliminated from the roots with normal growth. By raising the percentage of oxygen and keeping the gas agitated in the chamber, this result, however, was obtained. It was difficult to provide sufficient agitation of the gas and at the same time prevent desiccation of the roots. It was, probably, for this reason that it was found necessary to use a mixture of 75% oxygen and 25% nitrogen instead of the mixture of 50% oxygen and 50% nitrogen which had proved effective in water culture. The increase in length of roots in air and in the gas mixture was very similar, the length attained in 4 days in the former being 7.0–9.4 cm., and in the latter 7.0–10.0 cm.

From these results it appears that a percentage of oxygen optimum for growth will prevent or greatly retard air-space formation. It would seem, also, that any increase in air-space formation that may accompany rapid growth is due to the oxygen deficit arising from the increased respiration accompanying such growth and can be eliminated by providing the necessary extra oxygen. In agreement with this conclusion are the results of experiments on the effects of various temperatures using electrically regulated constant-temperature tanks. With other conditions as accurately controlled as possible, it was found that invariably a more rapid lacuna formation accompanied any rise in temperature, and consequent increase in respiration rate. At 15° C. or lower there were no spaces present after 4 days' growth in unaerated tap water, but they had made their appearance by the seventh day. At 20° C. or higher, they appear in most cases within 2 days and are large at the age of 4 days.

Although lack of oxygen unquestionably causes cell death and lacuna formation, a series of experiments indicated that oxygen of photosynthesis is not transported to the roots in sufficient quantity to affect this result. When other conditions remained constant, air-space formation was equally pronounced whether the plants grew in complete darkness, in alternating periods of light and dark, or under continuous illumination.

With respect to the mechanism by which scarcity of oxygen brings about death and collapse of the cells, three possibilities have presented themselves. In the first place, it is a recognized fact that in anaerobic respiration acids sometimes accumulate. A drastic increase in hydrogen-ion concentration might bring about significant changes in permeability or even in the chemical constitution of the protoplasm. Moreover, Cormack (1935) has shown that cells with a pH above 6.0 will have their

primary walls hardened with calcium pectate providing any calcium is available. Such cell walls do not collapse, even when the cells die, and under these conditions lacunae could not form. The truth of this statement was shown by experiments with the roots of peas and beans where a calcium pectate middle lamella is normal. These roots never have air spaces though placed under conditions where many cortical cells die.

Experiments, however, indicated that changes in pH were not a factor in the formation of cortical air spaces in the roots of *Zea Mays*. Roots grown in a series of solutions with readings from pH 3.8 up to well over 10.5 showed no significant difference in the size of air channels.

An investigation of the pH of the tissues themselves in a series of roots which formed air spaces and some which did not, provided evidence of a similar nature. The pH values were determined colorimetrically, using the method described by Brown (1924) for the determination of the pH of small quantities of liquid. The colours were made up carefully from dry powders, since it was found that the commercial colour solutions were too alkaline to differentiate between solutions not highly buffered.

In practice a piece of root 1.5 mm. long was used in each test. The test samples were cut, using a clean knife, at intervals of from one to three centimetres, depending on the length of the root. All units of the tip centimetre were, however, tested. A unit was carefully deposited in a drop of water (redistilled under glass) which was held in a chemically clean glass cell. A pestle was used for crushing the piece. It was made by drawing out a glass capillary tube and forming a bead on the end. This bead was flattened, squared and roughened by means of a file since the pieces of root were very hard to trap under a smooth surface. After thoroughly crushing the piece, enough redistilled water was added so that upon the addition of the drop of indicator (bromocresol purple) the surface of the liquid was flat and even with the top rim of the glass cell.

Precise measurements of the pH in the case of slight changes of pH from day to day are hardly possible, but the method is accurate enough to show any marked trend in the pH values along the length of the roots. Moreover, since vital dyes indicate little difference, if any, between the pH of the stelar and cortical tissue in corn roots, and also since the cortical tissue is, in volume, rarely less than 80% of the whole root, any marked change in pH must correspond to changes in the parenchyma of the cortex.

In all roots, whether producing air spaces or not, the pH gradually decreased in the first centimetre to a constant value. The pH at the tip varied from 5.5 in roots grown at high temperatures to 6.0 in low-temperature roots and fell to a value of 5.3–5.5 in both. No significant difference was to be seen in roots which formed spaces and those which did not.

It may be remarked here that by growing corn roots in a saturated solution of calcium diphosphate to which 5 cu. cm. of saturated calcium hydroxide solution per litre was added, the middle lamella of the cortical cells was changed to calcium pectate. Under these conditions lacunae were not produced.

A second possibility is that poisonous products of anaerobic respiration may act in other ways to destroy the protoplasm. Stoklasa (1908) has made careful chemical tests to detect the products of anaerobic respiration in Indian corn roots and has identified acetone, acetaldehyde and formic acid as being present under anaerobic conditions.

Thick longitudinal sections of roots were immersed for 1 hr. in various concentrations of these chemicals, then removed and tested for the presence or absence of living cortical cells. The percentages necessary to kill all the cells in 1 hr. are as follows: acetone 16 %; acetaldehyde 4 %; and formic acid 0.004 %. Quantitative tests to discover the amount of these substances in roots would be extremely difficult, but it seems obvious that formic acid is the only substance at all likely to occur in sufficient quantity to affect the life of the cell. From our own qualitative tests it seems improbable that there is enough, even of this substance, to kill the cells, although it may be present in sufficient quantity to produce a deleterious effect and so be a factor contributing to the final result.

The conception of anaerobic respiration obtained by Blackman (1928) from his experiments on apples suggests another possibility which, in the present state of our knowledge, seems to have more in its favour. In his schema the loss of carbon to the system during aerobic respiration is equal to that contained in the escaping carbon dioxide. At the same time, 3.0-3.5 times as much carbon is built back into the system through oxidative anabolism from the respiratory substrate. Thus, the major proportion of the substrate for respiration may go through the cycle time after time without being fully used up.

On the other hand, during full anaerobic respiration, the loss of carbon to the system is equal to that contained in the total amount of sugar which is glycolysed. The result is a much more rapid wastage of carbon in anaerobic respiration.

Should this schema be valid for Indian corn roots, there is every possibility of the normal respiratory substrate being used up more rapidly than it can be replaced. Under such conditions starvation would ensue and the cells would bring about their own destruction by degradation of substances necessary for the maintenance of vital organization.

In this connexion it may be noted that lacunae of the lysigenetic type were never observed in the stelar tissues, but only outside the endodermis. This fact may very well be explained by the proximity of this region to the conducting tissues which carry the food supply to the living cells.

An investigation of similar air spaces in the roots of three other members of the Gramineae, namely wheat (*Triticum vulgare* Vill.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.), disclosed an identical course of development to that already described for Indian corn. The only difference arose from the fact that a lesser concentration of oxygen in the medium was sufficient for the normal life processes of the cortical cells and, as a result, rapid aeration of the liquid in which the roots grew would prevent air-space formation.

SUMMARY

1. Indian corn roots under natural conditions produce large air spaces in their cortex.
2. These spaces are more irregular than those in *Sagittaria* roots, but their method of formation is the same.
3. The production of air spaces is preceded by deterioration and death of the protoplasm in groups of cells.
4. During this deterioration the cells lose their turgidity and their walls, being normally soft, due to the lack of calcium pectate, become crumpled and finally collapse.
5. Death of the cells has definitely been shown to be caused by scarcity of oxygen and a possible explanation for the action of this oxygen scarcity based on Blackman's conception of anaerobic respiration has been suggested.
6. Experiments with other species show different grades of susceptibility to oxygen scarcity and consequent differences in facility for producing air spaces.
7. In no case are air spaces produced when the pectic acid in the cell wall has been changed to calcium pectate.

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EXPLANATION OF PLATES I AND II

Transverse sections of *Zea Mays* roots grown in tap water

PLATE I

- Fig. 1. Section 115μ from root apex showing radial arrangement of cortical cells and small diamond shaped and triangular intercellular spaces. ($\times 211$.)
- Fig. 2. Section 6 cm. from the root apex. Large cortical lacunae, separated by irregularly arranged tissue consisting of walls of collapsed cells, are shown. ($\times 105$.)

PLATE II

- Fig. 1. Portion of Pl. I, fig. 1, enlarged. ($\times 570$.)
- Fig. 2. Epidermis and outer cortex at 505μ from root apex. Here a cell is shown separated radially from its neighbours leaving larger spaces. ($\times 570$.)
- Fig. 3. Portion of cortex at a level 1.5 mm. from the root apex showing a space due to cellular collapse. ($\times 570$.)
- Fig. 4. Portion of cortex 5.4 mm. from root apex. A large proportion of the cells show signs of approaching collapse. ($\times 570$.)
- Fig. 5. Epidermis and outer cortex 6 mm. from the root apex. A large space surrounded by collapsed cells is shown. ($\times 570$.)

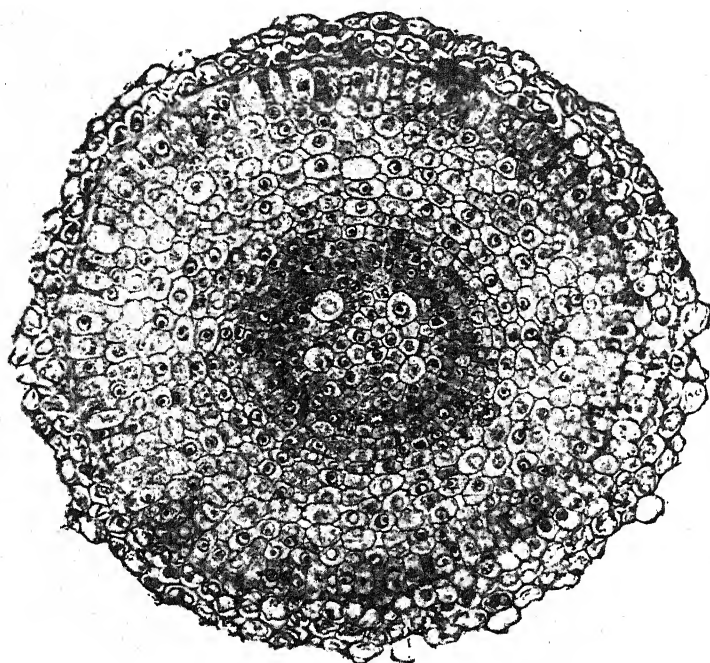


Fig. 1.

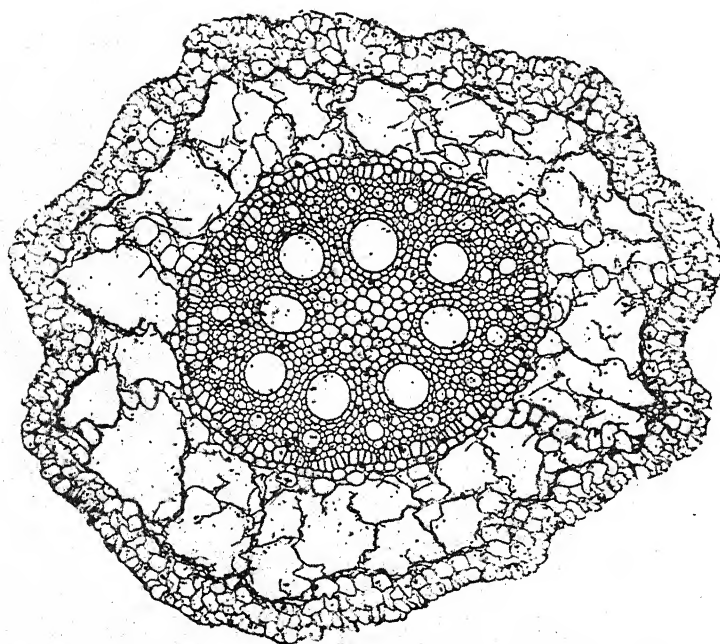


Fig. 2.

McPHERSON—CORTICAL AIR SPACES IN THE ROOTS OF *ZEAMAYL* L.

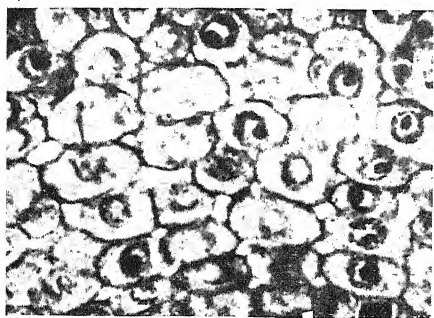


Fig. 1.

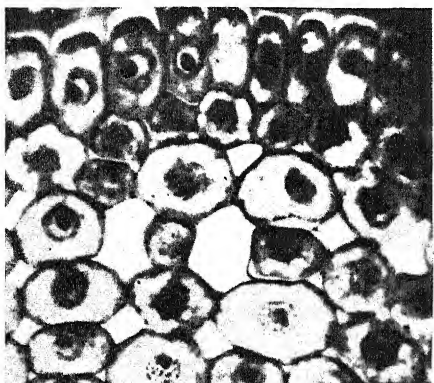


Fig. 2.

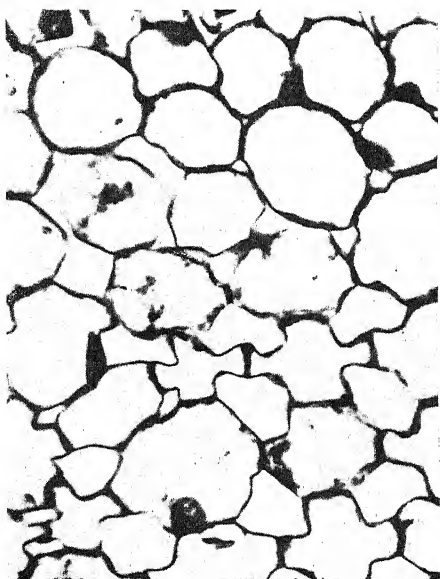


Fig. 3.

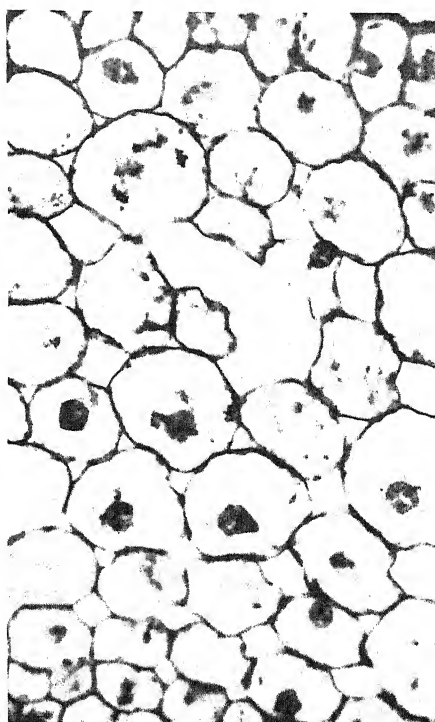


Fig. 4.



Fig. 5.

THE NEGLECT OF ANATOMICAL EVIDENCE IN THE CURRENT SOLUTIONS OF PROBLEMS IN SYSTEMATIC BOTANY¹

BY E. R. SAUNDERS
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TO-DAY the attention and interest of botanists is mainly concentrated on the species—the species from every point of view, though undoubtedly the botanical geneticist must, and does, concern himself ultimately with the individual. Employing the species, or it may be some larger group, as his working unit, the specialist in other fields than systematic botany relies on the systematist for the description which identifies his material. The systematist is the indispensable referee.

Now the systematist's description has hitherto in the main been simply a verbal picture of what is to be seen by inspection, a summarized statement of outward features. This has been perhaps natural. For the main end in view of the systematist such a description is ordinarily sufficient. But the picture thus outlined, though it may serve for the purposes of classification, is not in general presented in terms which reflect the relation between the superficial configuration and the underlying principles of floral construction. It depicts, but it does not analyse. It does not indicate *how* the particular conformation observed is to be interpreted in the light of the interrelations, physical and biological, governing the general ground-plan. The result is often to leave the impression that *any* structural arrangement is a possibility, with the further result that appearances have commonly been taken at their face value.

This position has doubtless come about because hitherto, except for one or two basic principles, there was little beyond outward form to rely upon. There was no systematized knowledge of floral anatomy. We knew, perhaps, in an overwhelming proportion of types all that appeared to be of importance to know about what I might refer to as the "flesh", but we had no extensive detailed knowledge of the "skeleton". We have now a certain amount of information on this latter subject, and one of the important facts which emerges from this new knowledge is that many structures are not what they appear to be and have till now been taken to be. Consequently, though the terminology hitherto employed by the systematist has in the main met practical needs, it is to some extent to-day out of harmony with reality. It often obscures the trend of evolution and the interrelations between allied forms. It has the further unfortunate result that it tends to induce or foster the frame of

¹ Being the full text of the paper read before Section K at the Meeting of the British Association for the Advancement of Science held at Cambridge in August 1938, of which a brief Abstract appears in the *Annual Report* of the Association for 1938.

mind which rests content with this type of description, and so dulls perception that there is anything more of importance to be learned about floral ground-plans which would carry us a stage farther towards the solution of the problems which they present. For it appears to me that nowadays there is, in general, whether or not in consequence, little appreciation of these problems. What *appears* to be is accepted. *How* it comes to be is not asked.

The following examples will serve to illustrate this viewpoint. In the genus *Cistus* the bulk of the species have pentamerous perianth whorls (K 5 C 5), but two (*C. ladaniferus* and *C. laurifolius*) are described as having only three sepals corresponding with the inner three of the pentamerous, quincuncial calyx of the majority, the two outer members having been lost. The same classification of species into two groups can be made in the allied genus *Halimium* and possibly others. Now among the species belonging to various genera, a series of grades in reduction of size of these outer sepals can be observed. It is therefore a ready supposition that in those species in which these sepals are not distinguishable they have been lost. But have they? In species of *Helianthemum* it can be seen that these small outer sepals, sepals 1 and 2 in the order of development, are gradually becoming adherent to the adjacent edges of the neighbouring sepals 4 and 5, respectively. What then has happened in those forms in which sepals 1 and 2 are presumed to have disappeared is that, in fact, coalescence with their neighbours has merely become complete. The two sepals are present, but they no longer attain independent morphological form. This could be easily proved, even if degrees of fusion did not occur, for the separate vascular system of both these sepals still persists. There is here then no real suppression, no absence. The failure is not in *development* but in *segmentation*.¹

The genus *Hypericum* offers another good illustration of a similar misinterpretation in a different floral whorl. The species in this genus have been grouped in the past into four classes—those with five antepetalous bundles of stamens and a pentamerous or a trimerous gynaecium, those with three bundles of stamens and a pentamerous or a trimerous gynaecium.

Now in the species having five bundles of stamens, each bundle admittedly corresponds with a single staminal member of an ordinary pentamerous type, which in the species in question has undergone multiplication. This has been assumed to be the case also in those species which have only three bundles, the two others being supposed to have disappeared. But, in fact, here as in the species with five separate bundles all five staminal members are represented. As in most Cistaceae, the sepals are arranged quincuncially. The result is that the space available for the development of the antepetalous whorl of the androecium (the only one present) is least on the radii of the two petals adjacent to the last sepal to develop, viz. sepal 5; it is next least on the radii of the two petals standing on each side of sepal 4; it is greatest on the radius of the petal standing between sepals 3 and 1. In consequence of this greater crowding on certain radii, the two vascular units proper to the two bundles of stamens bordering sepal 5 come into contact and become continuous, and the two bundles of stamens which they supply become one. Similar fusion takes place between the

¹ For a fuller description of the flower of Cistaceae see *New Phytol.* 35, pp. 47-67 (1936).

two vascular units, and between the two corresponding bundles of stamens which border sepal 4. Hence the difference in size (i.e. in the number of component stamens) between the three ultimate bundles, of which one alone corresponds to a single staminal member, each of the others representing two adjacent members.¹ But this relation is not to be discerned in the systematic descriptions, formulae or diagrams of the species making up the class having the stamens in three groups.

Veronica offers another and a particularly interesting instance simulating reduction in the number of floral members. Here too the perianth whorls in their fullest development are both pentamerous (K₅ C₅). But this condition is only attained in perhaps some half dozen species. Of the rest some have K₅ and a 4-lobed corolla and some K₄ and a 4-lobed corolla. Now in the calyx reduction from K₅ to K₄ can be traced through all gradations of diminution in size of the posterior sepal down to complete disappearance. Not so with the corolla, which almost always has either five normally developed lobes or four. When it is 4-lobed one lobe is posterior. That is to say this lobe lies on the same radius as the sepal which is in process of vanishing. The two are superposed. But since the vascular system of this sepal and of this superposed corolla lobe arise independently from the central vascular cylinder, such superposition is contrary to the fundamental principle of the alternation of successive whorls. This has been recognized. And it has been presumed in consequence that this posterior lobe of the corolla, though the apex is entire, must represent two petals completely united. This supposition is easily proved to be a fact, for in many such species, among them the common *V. arvensis*,² this posterior corolla lobe has two midrib bundles which give rise to two separate branch systems. In spite of the outward appearance to the contrary, five petals are represented in such types as *V. arvensis*, just as five sepals have been shown to be present in the two genera of Cistaceae cited above. But the systematist is ordinarily content to describe the corolla of such *Veronica* species merely as 4-lobed. The problem involved is passed by. The great value of the case of the *Veronica* corolla is that it demonstrates so clearly that segmentation—and it must be realized that the number of segments corresponds with the number of primordia at an earlier stage—is not invariably an infallible guide to the number of floral members. Yet, hitherto, the appeal has in general been to these two lines of evidence—to the number of primordia and the number of segments—and to these alone. Frequently they have not sufficed to solve the problem. In such case the problem, relegated to the background, is no longer regarded and has remained unsolved.

The several examples of congenital or induced fusion of floral members cited above are instances of fusion between, or non-segmentation of, some but not all of the members of one and the same whorl. Concrecence of a more complex nature occurs when more than one whorl is involved. Two instructive examples of this latter class are furnished by *Saraca* (Leguminosae-Caesalpinoideae) and *Soldanella* (Primulaceae).

¹ For further details see *J. Linn. Soc. (Bot.)*, 50, 313-20 (1936) and *Proc. Linn. Soc.*, Sept. 1937, pp. 160-3.

² For a fuller account of this and other species see *J. Linn. Soc. (Bot.)*, 49, 453-93 (1934).

(1) *Saraca indica*¹ has a coloured perianth of four segments which might at first glance be mistaken for a crucifer corolla slightly out of position. The systematist describes the flower as having a calyx of four coloured segments and as lacking a corolla. But how does a tetramerous calyx arise in a Caesalpinoid in which the floral construction is undoubtedly, from the evidence of the androecium, based on a pentamerous ground-plan? Inspection of the outward form, either in the mature stage or in the early stages of development, does not tell us. But the internal anatomy provides the clue. For in *Saraca*, as in the ordinary leguminous type with a pentamerous perianth of five sepals and five petals, ten vascular bundles turn out from the central cylinder at the flower base on equidistant radii and enter the four perianth segments. But whereas in the K₅ C₅ type one of these bundles enters each perianth member and becomes its midrib, in *Saraca* three of these bundles enter each of two opposite perianth segments, and two enter each of the other pair.¹ Clearly all of the ten perianth members present in the ordinary leguminous type are represented by the four segments in *Saraca*. Here, as in the simpler cases discussed above, there is no suppression of individual sepals or petals. There is no fundamental change in the vascular ground-plan, but merely an exceptional type of concrescence, of non-disjunction, of failure in segmentation, according as one may prefer to describe this modification in which two whorls are involved. Incidentally it may be noted that it is natural that the four perianth segments in *Saraca* should be coloured, for each must receive either one or two of the bundles which in the normal type belong to the corolla. Hence all four segments might be expected to show in some degree the characteristics of petals, as, in fact, they do.

(2) *Soldanella*, like other Primulaceae, has been regarded as conforming to the ground-plan K₅ C₅ A₀ + 5. The problem of the superposition of the single staminal whorl upon the petals in this Family has been discussed by numerous writers who have put forward almost every possible supposition in explanation of this apparent exception to the general rule of alternating whorls. Recent investigation has clearly shown that *none* of the assumptions of these writers is required, and that in fact the primulaceous ground-plan is in accord with the general principles underlying floral construction. For a survey of the Family makes it evident that the basic ground-plan is K₅ C₅ A₅ + 5, but that both staminal whorls only attain separate morphological expression in one or two genera, notably in *Samolus*, in which genus, however, the antesealous staminal members, though present, have already begun to degenerate, for their filaments are without anthers. That these structures do, nevertheless, represent a disappearing outer staminal whorl is clear from the fact that they are supplied with a vascular bundle which arises conjoined at first with a sepal midrib from which it later becomes detached, just as the antepetalous fertile stamens are supplied by bundles which arise conjoined with a petal midrib bundle and become free later. It has been shown elsewhere that when the vascular bundles for two superposed whorls originate from a common trunk cord, suppression of one of these two whorls will have no effect upon the radial position of the succeeding whorl.²

¹ For a fuller account of the flower *Saraca* see *New Phytol.* 28, 236 (1929) and *J. Linn. Soc. (Bot.)*, 49, 213, 1933.

² See *Floral Morphology; A New Outlook*, vol. 1, Introduction, p. 8 (1937).

Hence even complete suppression of the antesealous staminal whorl in Primulaceae would not affect the position of later whorls. No special explanation is therefore needed to account for the ground-plan in those types which lack separate antesealous staminal structures.

A further consideration to be taken into account in order to arrive at a correct interpretation of the "corolla" of *Soldanella* is that when sterilization of stamens occurs in a flower having a gamopetalous corolla and an epipetalous androecium, the normally unbranched vascular bundle of the stamen often becomes pinnately branched as in a vegetative foliar member. This assumption of (= reversion to) the foliar type of venation in sterile members of the androecium, accompanied by the assumption of foliar (petal) form and complete incorporation of these petal-like staminodes into the "corolla", is strikingly illustrated in this genus. Nevertheless, the "corolla" here, as throughout the Primulaceae, has hitherto been regarded as composed simply of five petals, although it evidently consists, in fact, of the five petals together with the five alternating (antesealous) staminodes, the two whorls, alike both in their outward form and in their venation scheme, having become welded into a single structure. In other Primulaceae the compound nature of the "corolla" is less obvious, owing to the fact that the midrib bundle of the antesealous staminal members comes to an end at the level at which the lateral veins arise. Associated with this mid-vein loss is the deep segmentation of the "corolla" in line with each lost midrib, thus splitting the antesealous staminal system in half as in a dichasium. Hence each of the five "petals" in reality represents one petal conjoined with half of the adjacent modified staminal member on each side. Hence, furthermore, the evidence of modification and incorporation into the corolla of the antesealous members of the androecium, obvious in *Soldanella*, is only revealed in other primulaceous genera by microscopic investigation.¹

Yet one more example where the truth has become obscured "in mists of hallowed error". It is a familiar fact that it is almost universal for the androecium in Cucurbitaceae, following upon a pentamerous calyx and corolla, to be represented by three anther-bearing structures, of which two appear from their double size to consist of two stamens showing in different types various degrees of coalescence up to complete fusion, each such pair standing in front of a petal. Whereas the third structure, a single stamen, stands a little out of line with another petal. These appearances readily lend themselves to the supposition that a complete pentamerous staminal whorl is, in fact, present and that this whorl is in reality antesealous, the antepetalous position of the two double structures having come about as the result of the convergence in two pairs of four out of the five members of the whorl. This interpretation takes no account of the anomalous position of the single stamen. In passing it may be noted that it is not always clear in systematic accounts whether the number of stamens stated to be present indicates merely the number of separate anther-bearing structures which can be observed with the eye, or the number of staminal members which the writer holds that these structures represent. But this

¹ For a fuller account see *New Phytol.* 33, 131-9 (1934); also earlier *Ann. Bot., Lond.*, 46, 260-81 (1932); and later *Floral Morphology*, 2, 406-12 (1939).

by the way. Now at a first glance the above explanation of the cucurbitaceous androecium ground-plan appears plausible and fairly satisfactory, but upon further consideration it is found to involve a serious difficulty, and one which, so far as I am aware, has been altogether ignored. This is brought out by the fact that in one genus, *Fevillea*, in addition to five fertile and similar stamens of which four are approximated in two pairs in the typical manner and obviously, therefore, correspond with the paired fertile stamens of other genera, there are present five staminodes which alternate with the petals. It is thus evident that the full androecium ground-plan must be represented as A5 staminodal, + 5 fertile, of which, however, only the fertile whorl is developed except in the above-mentioned genus. But current interpretation places this fertile whorl on the sepal radii. That is to say, according to this interpretation the developed fertile whorl arises on the same radii as the suppressed sterile whorl, a proposition which calls for thorough investigation before it is accepted. When the vascular ground-plan is examined we find that the vascular bundles for the fertile stamens do not arise on the sepal radii, but are carried out conjoined with part of the petal vascular systems. Instead, however, of being detached, as is usual in such circumstances, from the petal midrib bundles, they are detached from the primary pair of laterals derived from these midribs. The two approximated stamens forming a pair receive, respectively, the two laterals from the midrib of the petal in front of which they stand; the single stamen is served by one or other lateral of the midrib of the corresponding petal. Hence it is evident that the fertile stamens in all other Cucurbitaceae as well as in *Fevillea* represent members of an *antepetalous* whorl. Since their vascular system does not arise from the central cylinder independently, their radial position remains the same whether the antesepalous staminal whorl is developed or suppressed. Furthermore, since the single stamen, like the paired members, is served, not by the primary trunk cord originating on the petal radius but by a lateral from this cord, the slight deviation in the position of this member from the strict mid-line of the corresponding petal is natural and requires no assumption to account for it.¹

Having cited a number of instances to illustrate my contention that many current systematic accounts do not present a correct or full picture of the morphological nature of the structures described, I now come to the purpose for which I have drawn attention to this situation. My object is to make a strong plea for closer co-operation between the systematic botanist and the morphologist in order to facilitate the solution of problems which are common ground. I would stress the point that as those working in other fields must resort to the systematist for the critical determination of their material, so the systematist may find himself confronted with situations when his two sign-posts, number of segments and number of primordia, though useful as far as they go, prove to be not all-sufficing. These criteria do not serve, for example, to determine whether an apparent reduction in numbers is, in fact, a reality due either to suppression or to a change of ground-plan, in other words to a change of rhythm, or whether it is a deceptive appearance which results from coalescence, incorporation, synthesis or other complex process. Nor,

¹ For a more detailed account see *Floral Morphology*, 2, 489-97 (1939).

again, where there is an increase in number will this kind of evidence always serve to distinguish between multiplication due to an alteration in the ground-plan and multiplication resulting from the splitting or branching of members without change in the ground-plan. For the elucidation of all such forms of heteromery and of other problems of a like nature, the systematist needs to have recourse to the morphologist. With such co-operation it *should no longer* be possible, where structures are a constant feature of a Family and are situated on the primary construction radii in regular alternation with the preceding and succeeding whorls in the manner, for example, of the scales and teeth which alternate with the fertile stamens in *Amarantaceae*, and together with these stamens form a monadelphous androecium, to describe such structures as "having no morphological significance". With such co-operation it *should*, on the other hand, be possible to present a clearer picture of the mode of evolution of floral types in the past, and to provide an answer to at least one question raised by all floral variations—How?, though the separate and more fundamental enquiry—Why? may still have to await solution. As a first step towards such co-operation I suggest that it is essential that an endeavour should be made to attain uniformity in a terminology brought up to date. At present even within the ranks of systematists there is a lack of such uniformity, leading sometimes to ambiguity if not to contradiction, though not arising from any difference of view. I therefore conclude with an appeal that the two classes of observers, the field worker and the laboratory worker, should come to terms.

A SECOND FACTOR INVOLVED IN INHIBITION BY AUXIN IN SHOOTS

By R. SNOW

Fellow of Magdalen College, Oxford

(With 7 figures in the text)

1. INTRODUCTION

IN a previous paper the writer (1937) claimed to confirm the discovery of Le Fanu (1936), that the inhibiting effect exerted by a ring of "auxin paste" (that is, of hetero-auxin in lanoline) upon the parts morphologically above it in pea-shoots is quite different from any effect exerted by the paste on the parts below it. He further claimed to show that this upward inhibiting effect does not depend upon the morphological direction in which any substance or influence coming from the direction of the paste may be travelling in the inhibited parts, nor upon any obstruction of growth-promoting substances coming up from roots or cotyledons. The latter two conclusions were drawn from the experiment illustrated in Fig. 1 which is reprinted from the previous paper: for in this experiment the auxin paste was found to inhibit *both* halves of the young split internode just above it, as well as the internode next younger again. A similar inhibition was found with the arrangement shown in Fig. 2*a*, but with the arrangement of Fig. 2*b* the parts above the paste were unexpectedly found not to be inhibited at all. This last result was left unexplained, and some attempts to find the explanation will be reported in the present paper.

2. MATERIALS AND CONDITIONS

The pea seedlings, of race "Thomas Laxton", were grown in soil in a greenhouse, but screened from direct sunlight. The hetero-auxin crystals used were supplied by the firm of Fraenkel and Landau of Berlin, and had been, when new, the best sample out of several which were obtained from different firms and were found to differ greatly in activity. But in the present experiments the crystals were 16 months old and had lost about half of their activity, as was shown by tests on oat coleoptiles, though kept in an airtight tube and in a refrigerator. The hetero-auxin was applied as before in lanoline made from equal quantities of wool-fat and water.

3. THE EFFECT OF THE TRANSPIRATION STREAM

It might perhaps be suggested that the failure of the hetero-auxin in lanoline, or "auxin-paste", to inhibit the parts above when applied to the base of a downward-pointing strip of stem, as in Fig. 2*b*, was due to the fact that the transpiration stream was reversed in the part of the strip above the paste. So the experiment illustrated in Figs. 2*a*, *b* was repeated, but with the modification that the extreme bases of the

downward-pointing strips were allowed just to dip into water and to draw it up from tubes placed beneath them. To make this possible, the paste, when applied round the lower end of the downward-pointing strip, was kept just away from the extreme base. The strength of the paste was for some of the plants of each set 1 in 500 (= about 1 in 1000 with fresh hetero-auxin), and for others 1 in 300

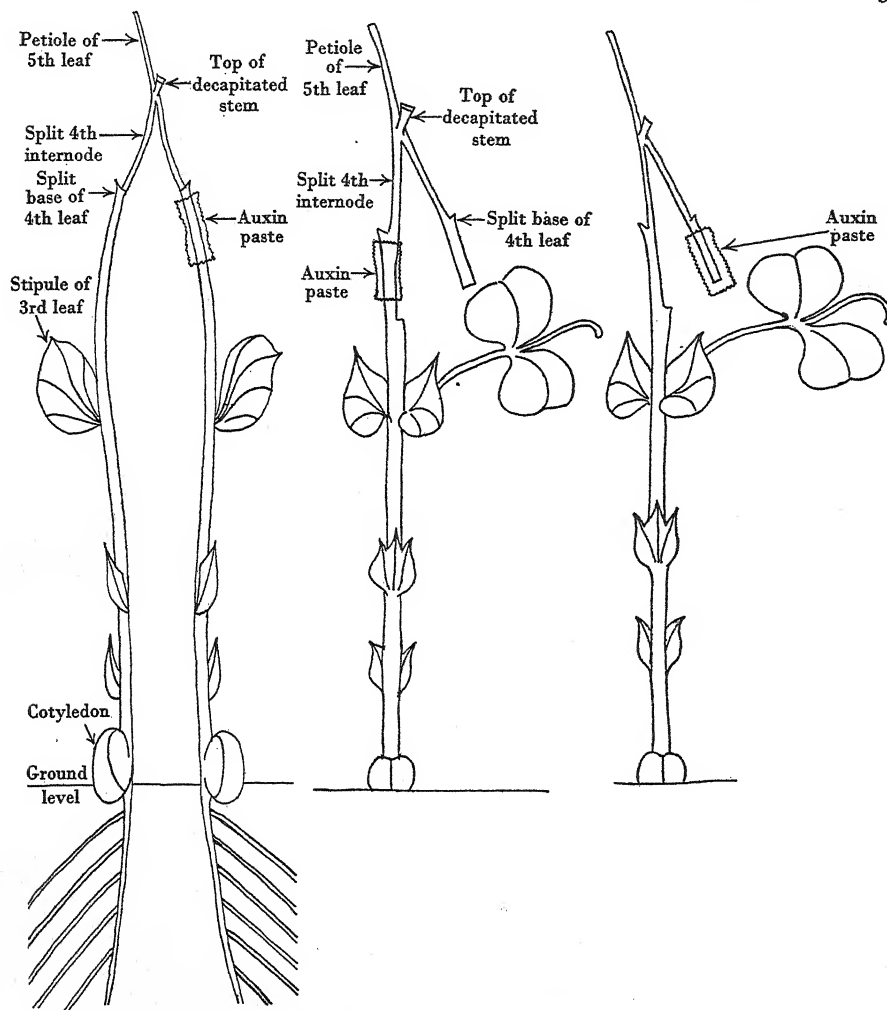


Fig. 1

Fig. 2a

Fig. 2b

(= 1 in 600 fresh). The result with both pastes was the same as before—strong inhibition of the internodes above the paste when it was applied as in Fig. 2a, and none when it was applied as in Fig. 2b. It was also noticed that the axillary bud at the node next but one above the paste was strongly inhibited with the former arrangement and not at all with the latter (the bud immediately above the paste was destroyed by the split).

However, this experiment is not conclusive, since the transpiration stream may still have been reversed in the downward-pointing strips, if the resistance to drawing up water from the roots and down the strips was at any time less than the resistance to drawing it up through the strips from the tubes below. So the following experiments were performed (Exps. 1 and 2, Figs. 3 and 4), the idea being to make, first, an arrangement similar to Fig. 2*a*, but with the transpiration stream reversed above the paste, and, secondly, an arrangement similar to Fig. 2*b*, but with the stream certainly not reversed above the paste.

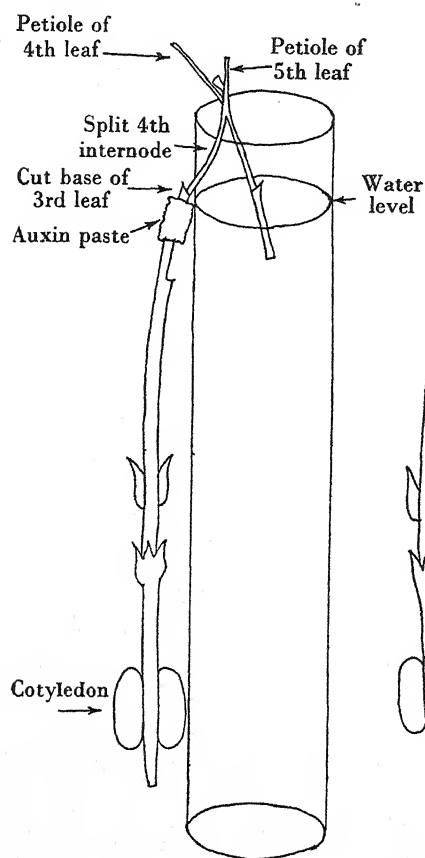


Fig. 3

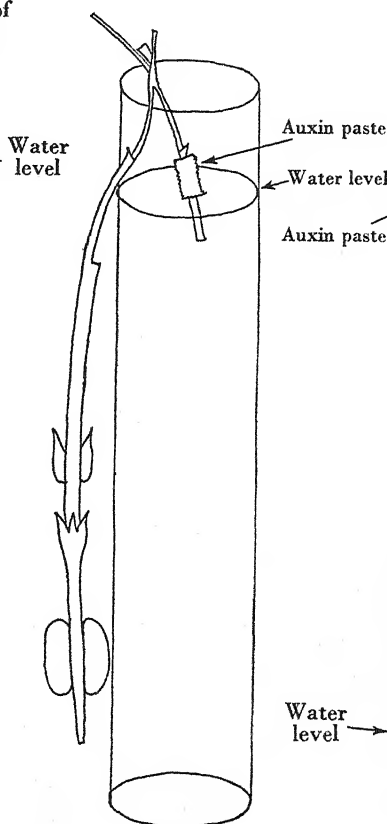


Fig. 4

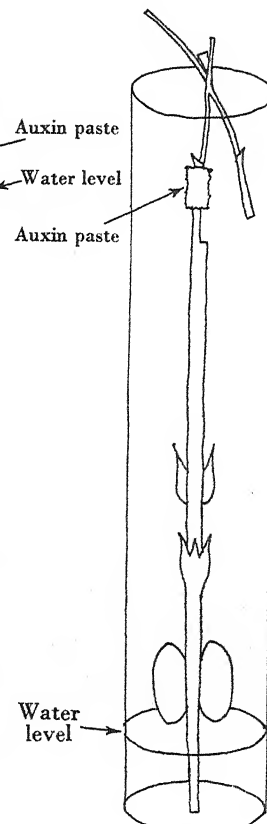


Fig. 5

In Exp. 1 (Fig. 3) the arrangement was similar to that of Fig. 2*a*, with the paste on the main part of the stem, except that the pea seedlings were deprived of their roots and were hooked on to the rims of tubes containing tap water, in the manner illustrated, so that they drew up their water from the tubes through the downward-pointing strips of stem, and the transpiration stream was reversed in the half of the split internode (the 4th)¹ just above the paste. They were kept before a window and

¹ In numbering the internodes from below upwards, the epicotyl will here and in future be counted as being itself the first internode, though in the writer's previous papers it was not so counted.

allowed to transpire slowly, but were not exposed to direct sunlight: the water was kept up to the level shown, and the cut surfaces not submerged were vaselined. The concentration of the paste was 1 in 500 (= about 1 in 1000 with fresh hetero-auxin). Controls were treated similarly, except that they received no auxin paste. The results are given in Table I.

Table I, Exp. 1 (see Fig. 3)

		At start, length in mm. of 4th internodes	After 12 days, lengths in mm.			
			4th internodes		5th internodes	6th internodes
			Main half	Half on downward- pointing strip		
Plants with auxin paste: No. 1		5.5	16.5	9.5	17	4.5
2		7.0	13.5	12.5	8	<2
3		5.5	14.0	10.5	13	<2
4		5.5	12.0	10.0	15	<2
5		5.0	12.0	10.0	13	<2
Mean		5.7	13.4	10.5	13.2	<2.5
Controls	No. 1	6.0	19.0	13.0	38	13.0
	2	6.5	18.0	12.5	27	12.5
	3	5.5	19.0	12.5	35	12.5
	4	4.5	16.5	11.5	36	11.5
	5	5.0	16.0	9.5	31	9.5
Mean		5.5	17.7	11.8	33.4	11.8

Table I shows that the 5th and 6th internodes, which last were at the start too small to measure, were strongly inhibited by the auxin paste below, although they were separated from it by the half of the split internode in which the transpiration stream was reversed. This half of the 4th internode was also itself slightly inhibited. It follows that an upward-moving transpiration stream is not necessary to enable the paste to inhibit the parts above, and consequently that the failure of the paste to inhibit when applied to a downward-pointing strip, as in Fig. 2*b*, cannot be entirely explained by the absence of such a stream. In this respect the upward inhibition by auxin paste resembles natural correlative inhibition by growing shoot apices, since correlative inhibition can travel up a side-shoot in which the direction of the transpiration stream is reversed (Snow, 1937, p. 286).

But it remains possible that an upward-moving transpiration stream, though not necessary for upward inhibition, may yet be a factor favourable to it, and accordingly this possibility was tested by Exp. 2 (Fig. 4). In Exp. 2 the pea plants were operated upon as before, being again deprived of their roots and hooked on to the rims of tubes containing water; but the auxin paste (the same paste) was put at the corresponding level round the downward-pointing strip of stem. Below the paste a length of about 10 mm. was left to dip into the water and to draw it up to supply the plant. Thus the paste was in the same position as in the experiment shown in Fig. 2*b*, but the transpiration stream was not reversed in the zone above the paste.

Controls were treated similarly, but received no paste. The results are given in Table II.

Table II, Exp. 2 (see Fig. 4)

	At start, length of 4th internodes in mm.	After 11 days, lengths in mm.			
		4th internodes		5th internodes	6th internodes
		Main half	Half on downward pointing strip		
Plants with auxin paste: No. 1	7.5	15	11	29	3
2	7.5	13	10	39	9
3	6.5	9	8	32	6
4	6.0	13	9	35	5
Mean	6.9	12.5	9.5	33.7	5.7
Controls: No. 1	6.5	14	14	41	11
2	7.0	15	12	34	24
3	6.0	14	15	36	14
Mean	6.5	14.3	13.6	37	16.3

Table II shows that in this experiment (Exp. 2, Fig. 4) there was a little upward inhibition, but much less than in Exp. 1. For the 5th internodes (the next but one above the paste) were not significantly inhibited at all, but the still younger 6th internodes were considerably inhibited, and the halves of the split 4th internodes immediately above the paste were also slightly inhibited. So although an upward-moving transpiration stream is not *necessary* for upward inhibition, it does appear to help a little towards the bringing about of the inhibition—unless indeed the difference between the result of the present experiment and that of the previous experiment shown in Fig. 2*b* was due to the fact that in the present experiment a length of 10 mm. of stem had to be left below the zone to which the paste was applied.

This conclusion is supported by the result of Exp. 3 (Fig. 5), when compared with the result of Exp. 1. In this experiment the intention was to make the arrangement and conditions as similar as possible to those of Exp. 1, with the paste on the main part of the stem, except that the transpiration stream was to move upwards in the main stem, instead of being reversed. So the plants were operated upon just as in Exp. 1, and the paste was applied to the same zone of the main part of the stem; but a stump of about 20 mm. of the main root was left, and the plants were placed with this root stump dipping into a little water in the base of a tube. All lateral roots were removed, since the presence of elongating and functional roots would have been a complicating factor and would have spoilt the comparison with Exp. 1. The results are given in Table III.

Table III shows that the upward inhibition in Exp. 3 (Fig. 4), with an upward-moving transpiration stream, was slightly stronger than it was in Exp. 1 with the transpiration stream reversed. So this result also indicates that in these conditions

Table III. Exp. 3 (see Fig. 5)

	At start, length in mm. of 4th internodes	After 11 days, lengths in mm.			
		4th internodes		5th internodes	6th internodes
		Main half	Half on downward- pointing strip		
Plants with auxin paste: No. 1	6.5	9	8.5	3	< 1
2	7.0	11	12.5	21	1.5
3	5.5	10	11.5	17	1
4	7.0	12	10.0	21	1
Mean	6.5	10.5	10.6	15.5	1
Controls: No. 1	6.5	14	12.0	38	5
2	6.0	16	13.5	44	7.5
3	5.5	21	16.0	52	15
4	7.5	20	15.0	35	5
Mean	6.4	17.7	14.1	42.2	8.1

an upward-moving transpiration stream helps a little, but only a little, towards bringing about upward inhibition. This is not surprising, since it is known that auxin solutions, if introduced into the transpiration stream even in low concentrations, inhibit the young parts of the shoot, and the lateral buds (see Le Fanu, 1936, p. 207; Snow, 1936, p. 301). So presumably when the auxin is applied in lanoline, a little of it leaks into the transpiration stream and produces a similar effect. But the greater part of the upward inhibition caused by auxin paste must be brought about in some other way, as is shown by Exp. 1 when compared with Exps. 2 and 3.

4. THE EFFECT OF THE PARTS BELOW THE AUXIN PASTE

The above experiments make it clear that the failure of the auxin paste to inhibit when applied to the basal end of a downward-pointing strip of stem, as in Fig. 2*b*, cannot be due, for the main part, to the reversal of the transpiration stream, and consequently some other explanation must be sought. The results so far obtained suggest that the presence of a length of leafy shoot and of roots and cotyledons (or of some one of these three) below the auxin paste may somehow be necessary to enable the auxin to bring about the reactions which result in the upward inhibition; and this possibility can be further tested by experiments not involving the cutting out of downward-pointing strips of stem. It would not indeed be of any use for this purpose to compare the effects of applying rings of auxin paste near the tops and near the bases of pea shoots, since rings of the auxin paste applied to the older zones of stem nearer the base do not inhibit the zones above, nor the lateral buds, as the writer has found. This is presumably because the auxin does not penetrate from the sides in the older zones, since the same paste applied to the upper cut surface of a shoot decapitated near the base inhibits the lateral buds strongly. Van Overbeek

(1938, p. 154) has reported similar results. So to be effective the rings of paste must be applied to young zones of stem, preferably not more than 8 or 10 mm. below the apex, as in the previous experiments. The following experiment is roughly what is wanted.

Exp. 4. Pea seedlings, with their 5th internodes about 6 mm. long, were divided into four groups, *a*, *b*, *c* and *d*. Group *a* were given rings of the same auxin paste, 1 in 500 (=about 1 in 1000 fresh), just below the young 5th internode (Fig. 6). Group *b*, without auxin paste, served as controls to *a*. Group *c* were given rings of

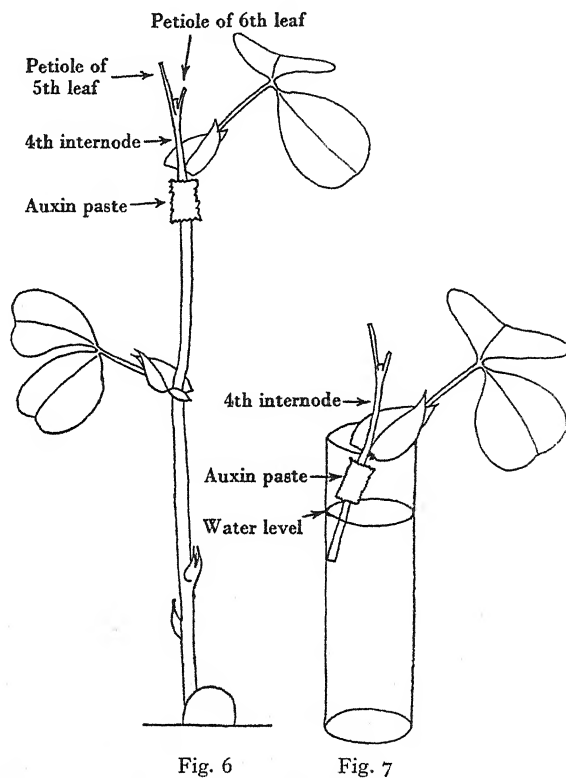


Fig. 6

Fig. 7

the same paste as *a* in the same position, but then the upper parts of their shoots, which were the parts to be observed, were cut off at a level only from 8 to 20 mm. below the paste, and were placed as cuttings with their bases in a little water (Fig. 7). These cuttings had above the paste one leaf (the 4th) which was just expanded and served for photosynthesis. Group *d*, without paste, were controls to *c*. In all groups the young leaves near the apex were removed, since these tend to counteract the upward inhibition by the paste below (Le Fanu, 1936). All groups were kept in a greenhouse (in March) and lightly screened from direct sunlight: the cuttings were protected against excessive transpiration. The purpose was to compare the upward inhibition in group *a* with that in group *c*. The results of this and of another similar experiment carried out in June are given in Tables IV and V. In the later

experiment the plants were a little older, so that the young internode beneath which the paste was put was the 6th.

Table IV. Exp. 4 (see Figs. 6, 7)

	At start, mean length in mm. of 5th internodes	After 8 days, mean lengths in mm.	
		5th internodes	6th internodes
Group <i>a</i> (4 plants)	5.0	27.7	1.5
Group <i>b</i> (5 plants)	5.0	65.6	14.9
Group <i>c</i> (5 cuttings)	6.1	15.2	2.9
Group <i>d</i> (5 cuttings)	6.1	17.8	4.7

Table V. Exp. 5 (see Figs. 6, 7)

	At start, mean length in mm. of 6th internodes	After 6 days, mean lengths in mm.		After 9 days, mean lengths in mm.	
		6th internodes	7th internodes	6th internodes	7th internodes
Group <i>a</i> (3 plants)	5.5	44	5.0	57	16.8
Group <i>b</i> (3 plants)	5.2	88	22.3	104	64.0
Group <i>c</i> (4 cuttings)	5.8	—	—	22.1	4.1
Group <i>d</i> (5 cuttings)	5.9	—	—	25.1	3.3

In Tables IV and V one can see, by comparing the growth in groups *a* and *c* with that in the corresponding control groups *b* and *d*, that there was strong upward inhibition in the rooted plants of group *a*, but practically none in the cuttings of group *c*. This was in spite of the fact that the rooted plants without paste (group *b*) grew much faster than the cuttings without paste (group *d*), and that as a general rule rapidly growing buds and shoots are *less* easily inhibited than slowly growing ones. The results therefore agree with the previous experiments in showing that the auxin paste applied to a young zone at or near the base of a length of stem scarcely inhibits the parts above at all, although it inhibits them strongly if it has below it the usual other parts, stem, leaves, cotyledons and roots. It follows that some of these parts when present below the paste must greatly increase its inhibiting effect on the parts above, and the manner in which they do so will be discussed in § 6. However, it will be shown in § 5 that a stronger paste can inhibit to some degree even when applied to the base of a length of stem.

5. FURTHER EXPERIMENTS ON THE EFFECT OF THE PARTS BELOW THE AUXIN PASTE

The question remains, *which* of the various parts below the auxin paste enable it to inhibit the young parts above? The following experiment shows that a length of stem carrying one leaf is enough to do so, even without any roots.

Exp. 6. Shoots of pea seedlings were given rings of the same auxin paste at the same level as in the last experiment (see Fig. 7), but their upper parts were cut off

not close below the paste but lower down, so that there remained below the paste about 8 or 10 cm. of stem with one mature leaf, in addition to the mature leaf just above the paste. The cuttings were exposed to diffuse light (in March) with their bases in water. Similar cuttings without paste served as controls.

At the start the mean length of the young internodes just above the paste in four cuttings was 6.1 mm., and that of the corresponding internodes in four cuttings without paste 6.9 mm. After 8 days the mean length of these internodes in the cuttings with paste was 20.1 mm. and in those without paste 54 mm., while the mean length of the next younger internodes was 3.2 mm. in the former and 11.2 mm. in the latter. Thus the internodes above the paste were inhibited about as strongly in these cuttings with one leaf below the paste, as they were in the rooted plants of Tables IV and V.

Incidentally, it may be noted that in these and other pea cuttings, whether supplied with auxin or not, the fully elongated parts of the stem became after some days curiously stiff, whereas in the rooted plants they remained flexible.

So far as concerns the inhibition of lateral buds by auxin paste placed on top of a decapitated shoot, the following experiment shows that cotyledons below the paste, together with only a very short length of axis, are also enough to enable the paste to inhibit with full strength, and reasons will be given in § 6 for thinking that this inhibition of lateral buds is the same process as the upward inhibition in the main stem.

Exp. 7. Pea seedlings were decapitated in the epicotyl only 10 mm. above the cotyledons, and were given caps of the same auxin paste on the tops of the stumps. Some of them were left rooted in the soil; others were dug up, and had their main roots cut through at about 12 mm. below the cotyledons and all lateral roots removed. The latter seedlings were then arranged as cuttings with their root stumps in tap water. Each set of seedlings was matched with a corresponding set of controls which were given vaseline instead of auxin paste.

After periods of from 7 to 10 days (in May) the mean growth of eight axillary buds of the cotyledons in four rooted plants with paste was 1.25 mm., and in four control plants 6.2 mm. Meanwhile in four cuttings with paste the mean was 1.1 mm., and in four control cuttings 6.9 mm. So the buds were inhibited as strongly in the cuttings as in the rooted plants.

The auxin paste also inhibits the lateral buds strongly when applied to the tops of very short cuttings carrying each a single leaf, as the following experiment shows.

Exp. 8. From pea shoots cuttings were taken comprising one node with its leaf (the youngest expanded leaf but one), and 5 mm. of stem above the node and from 10 to 15 mm. of stem below it. Some of the cuttings were given lumps of the same auxin paste on their upper cut surfaces, and others, with vaseline instead of auxin paste, served as controls. In this and in the subsequent experiments with single-leaf cuttings, the paste was applied so that it did not overflow on to the sides of the stem, or not for more than 1 mm. at most. The cuttings were kept in diffuse light.

After 7 days (in June) the mean growth of the axillary buds in six cuttings with auxin paste was 0.7 mm., and in six cuttings without paste 7.6 mm.

From Exps. 6-8 it is clear that a length of axis together with a pair of cotyledons or a single leaf below the auxin paste is enough (in the light) to enable the paste to inhibit strongly; and, further, that it is enough even when the length of axis is very short, 20 mm. or less. But a short length of bare stem, of 15 or 20 mm., does not have this effect (Exps. 4, 5). Whether a longer stretch of bare stem or a root system has this effect has not been determined.

It might be thought that the effect of the parts below the auxin paste on inhibition could be tested most simply by comparing the inhibitions of lateral buds caused by paste applied to the morphologically upper and lower cut surfaces of cuttings. But the objection is that the auxin probably penetrates much less easily from the lower cut surface, since it is only feebly transported upwards; and this spoils the comparison. Nevertheless, it is of some interest to note the effect of putting auxin paste on the basal ends of cuttings, as was done in the following experiments.

Exp. 9. Single-node cuttings were taken similar to those of the last experiment, except that the leaf was the youngest fully expanded leaf, and that they had about 25 mm. of stem left above the node and 15 mm. below it. They were placed inverted with their morphologically upper ends in tap water, and lumps of the same auxin paste were placed on their basal cut surfaces.

After 9 days (in June) the axillary buds had grown in four cuttings with paste: mm. 2.0, 1.75, 1.75, 1.0 (mean = 1.6) and in four control cuttings: mm. 2, 2, 1.75, 1.75 (mean = 1.9). Thus the buds were not inhibited by the auxin paste when applied to the bases of the cuttings. Another similar experiment gave a similar result.

However, a stronger paste inhibited the buds fairly strongly even when applied to the bases of cuttings, as follows.

Exp. 10. Inverted single-node cuttings, similar to those of the last experiment, were given lumps of auxin paste of strength 1 in 150 (=about 1 in 300 fresh) on their basal cut surfaces. After periods of from 7 to 9 days (in May), the mean growth of the axillary buds in five cuttings with paste was 1.45 mm., and in five control cuttings 5.75 mm.

The results of the last three experiments confirm those of Le Fanu (1936), who applied hetero-auxin in gelatine to the apical or basal cut ends of similar pea cuttings, erect or inverted, and found that the auxin inhibited the buds from either cut end, but more strongly from the apical end. The difference in her experiments between the effects exerted from the two ends would indeed have appeared still greater if she had had two sets of controls, one set erect and one inverted, instead of only an inverted set.

How is it to be explained that in Le Fanu's experiments and in the last experiment here reported a sufficiently strong source of auxin caused inhibition even when applied to the base of a length of stem? The answer may be simply that the presence of leaves or cotyledons below the paste is not absolutely necessary for inhibition, but only increases the strength of the inhibition very greatly; and that in the experiments with the weaker paste here reported the inhibition was not strong enough to be detected (or scarcely so) unless leaves or cotyledons were present below. It is also possible that in the experiment with the stronger paste (*Exp. 10*)

the single expanded leaf, which was in a position morphologically above the paste, may have acted in the same way as a leaf below the paste, though less effectively. In the experiment with the weaker paste, the leaf above the paste, when present alone, as in Exp. 9 and in the cuttings of Exps. 4 and 5, was not enough to make possible an inhibition strong enough to be detected.

It should perhaps be pointed out that when solutions of auxin are drawn up with the transpiration stream from the cut bases of shoots, the conditions are very different and the results cannot easily be compared.

6. THE RELATION BETWEEN UPWARD INHIBITION BY AUXIN PASTE AND CORRELATIVE INHIBITION

The opinion that upward inhibition by a ring of auxin paste applied to the main stem is the same process as the natural correlative inhibition of lateral buds and shoots has already been expressed by Le Fanu (1936, p. 217), who discovered the former effect, and by the writer (1936, p. 300). The following evidence is available.

First, Thimann & Skoog (1934) showed that auxin applied in agar to the upper cut surfaces of decapitated pea shoots inhibits the lateral buds below. They gave convincing grounds for considering that this inhibition was similar to the correlative inhibition of the lateral buds by the growing leaves near the apex, which are known to form auxin. The auxin may also be applied to the cut surfaces in lanoline as a paste.

Secondly, rings of auxin paste, applied round young internodes of shoots decapitated close behind the apex, also inhibit the lateral buds below, as the writer has found, though in the concentrations which he has used they do not inhibit them completely¹: and it is these same rings of paste which inhibit at the same time the young internodes of the main shoot above them. They also inhibit lateral buds above them, as was mentioned in § 1. If the shoots have not been decapitated, then these rings of auxin paste inhibit the young leaves of the terminal bud (Snow, 1936, p. 299).

All these inhibitions are covered by Le Fanu's rule (1936, p. 217) that auxin in shoots inhibits those parts which it approaches from a position morphologically below them (since the auxin in the main stem necessarily approaches the lateral buds from their bases), and there are no grounds for thinking that these inhibitions differ in nature.² The only difference which might be suggested is a difference between inhibition of shoots and inhibition of buds. But there is no sharp distinction between buds and shoots, and furthermore, inhibition of shoots involves inhibition of buds, since the inhibited shoots themselves possess terminal and lateral buds, which are also inhibited. There are therefore no grounds for doubting that upward inhibition of shoots by rings of auxin paste is the same process as inhibition of lateral buds by

¹ Le Fanu (1936, p. 210), on the other hand, found unexpectedly that the lower lateral buds were made to grow out by a ring of auxin paste applied higher up to undecapitated pea shoots. Perhaps this was somehow due to the fact that, besides applying a ring of paste, she also split the shoots and inserted the paste into the split. It is quite the opposite to what the writer has found.

² A more accurate way to express Le Fanu's rule is that auxin tends to inhibit those parts of shoots which it cannot reach by travelling in the morphologically downward direction (see Snow, 1938, p. 181). The rule may not always apply to solutions of auxin introduced into the transpiration stream.

sources of auxin applied to the main stems, and the same also as natural correlative inhibition. Consequently the conclusions reached concerning the nature of upward inhibition by rings of auxin paste will apply to natural correlative inhibition also.

7. CONCLUSION

How is it to be explained that various parts, including leaves and cotyledons, when present below the zone to which the auxin paste is applied, greatly increase the upward inhibiting effect of the paste? These parts do not enable the auxin to penetrate more easily, nor apparently do they cause it to travel upwards any more easily: for the swellings caused by the paste were about equally large and extended equally far (about 1 cm.) above the paste even when it was applied to the basal ends of strips of stem, although it then caused practically no inhibition (see Snow, 1938, p. 184). So the only conclusion which remains possible is that the parts below the paste somehow enable the auxin to *act* more effectively in causing inhibition. This makes it probable that the auxin descending the stem reacts or co-operates there with some factor supplied by the leaves and cotyledons in such a way as to cause indirectly the inhibition. The results therefore tend to support the explanation advocated previously by the writer for correlative inhibition (1937, p. 294), according to which the inhibition originates indirectly from some reaction promoted by the auxin in the main stem.

If this explanation is correct, the following three factors at least are concerned in causing the inhibition: first, the auxin in the main stem, secondly, the factor supplied by leaves or cotyledons with which the auxin reacts or co-operates, and thirdly, the inhibiting factor which originates from this reaction or co-operation and travels upwards into parts such as lateral buds and shoots or the region above the auxin paste, where the auxin, moving mainly downwards, cannot easily follow it. The word "factor" is here intended, in the widest sense, to include either a substance or an influence of some other kind. Also the terms "main stem" and "main shoot" are to be understood physiologically in the sense of "dominant"; for it is possible to make a morphologically lateral shoot dominant, so that it then inhibits the morphologically main shoot (Mogk, 1913). The dominant shoot will in general be the one down which most auxin is travelling (see Snow, 1931, 1937, p. 297).

8. NOTE ON A RECENT THEORY OF INHIBITION

Van Overbeek (1938) has proposed an interesting theory of inhibition. He rejects the "direct" theory and Went's "diversion" theory, though his grounds for rejecting the latter do not seem very strong (p. 160). He suggests that the auxin in the main stem finds its way into some conducting cells, probably the sieve tubes, in the bases of the lateral buds, and somehow prevents these cells from functioning so as to supply the buds with substances necessary for growth. In the main stem the auxin is supposed not to obstruct the sieve tubes in this way, because there they are much wider.

But clearly his theory in this form, like Thimann's "direct" theory (1937), could

only explain the inhibition of lateral *buds*, whereas actually the growth of quite long shoots, including the elongation of their internodes, can be inhibited correlatively, as was pointed out before (Snow, 1937, p. 284). The elongating internodes of pea shoots can also be inhibited by rings of auxin paste placed just below them (Le Fanu, 1936; Snow, 1936, 1938). Van Overbeek does indeed report that even a strong hetero-auxin paste (1 in 100) applied to the top of a decapitated pea stem could no longer inhibit the lateral buds if applied 3 days after the decapitation, when the buds had begun to grow out. This result is certainly in accordance with his theory, but the facts mentioned above remain and cannot be explained by it. Also van Overbeek's theory cannot explain the nearly opposite effects exerted by auxin paste on young internodes according as it is applied above them or just below (Le Fanu, 1936; Snow, 1938): nor can it explain how it is that the terminal bud of an intact shoot is not inhibited by its own auxin.

Nevertheless, the suggestion that auxin in some special cells, perhaps the sieve tubes, may travel upwards and may tend to inhibit growth is worth considering, especially since Hüber and others (1938, p. 1035) have lately found a lot of auxin in the sap of sieve tubes of trees. But it would be more plausible to explain the fact that the main stem is not inhibited as being due to the protecting influence of the main stream of auxin travelling down it in other cells (see Snow, 1937, p. 297). In this way one could also get over the other difficulties mentioned above.

But even so the theory would not fit in well with the results of the present paper, and it would be necessary to show that inhibited side-shoots contain auxin in some bound condition even in their upper parts: for Le Fanu (1936) and the writer (1937) have shown that they do not contain freely diffusing and growth-promoting auxin in appreciable amounts. Van Overbeek's evidence of upward transport in side-shoots (p. 160) seems insufficient, since, first, the distance of transport was very small, and secondly, auxin may perhaps be transported upwards more easily in the dark, in which his plants were grown.

Van Overbeek's statements (p. 160) concerning the "indirect" explanation of inhibition advocated by the writer (1937) contain several errors. For the writer did not assume that the secondary inhibiting influence was an inhibiting substance, though he gave reasons for thinking that probably it was (1937, pp. 295, 298): he did not assume that the primary process in the main stem from which the inhibiting influence originated was necessarily a growth process (p. 295): he did not assume that auxin in pea shoots is unable to travel morphologically upwards, but concluded from experiments that it can travel upwards, but only feebly (pp. 286, 294).

Ferman also (1938) has proposed a theory of inhibition which is rather similar to Went's "diversion" theory: but discussion of it must be postponed.

9. SUMMARY

1. An attempt is here made to find the explanation of the fact previously reported (Snow, 1938), that in pea shoots auxin paste applied to the basal end of a downward-pointing strip cut out from a young zone of the stem, as in Fig. 2*b*, scarcely inhibits the parts above at all, although it inhibits them strongly when

applied to a similar young zone which is connected directly with the base of the plant, as in Figs. 2a, 1. From consideration of Fig. 1 it is clear that the explanation cannot be that the inhibition depends on an obstruction of substances coming from the base of the plant (see Snow, 1938, p. 181).

2. Neither can the explanation be merely that the transpiration stream is reversed in the downward-pointing strip: for it is shown in § 3 that an upward-moving transpiration stream is not necessary for upward inhibition by auxin paste, though it does appear to increase the strength of the inhibition a little.

3. By experiments on cuttings of pea shoots compared with rooted plants, it is shown in §§ 4 and 5 that auxin paste inhibits much less strongly, if at all, when applied near the basal end of a length of stem. But if a mature leaf or a pair of cotyledons is present below the zone to which the paste is applied, then the paste inhibits with full strength. These results explain for the main part how it is that the paste does not inhibit, or scarcely at all, when applied to the base of a downward-pointing strip, as in Fig. 2b. The reversal of the transpiration stream may also contribute to this result in some lesser degree.

4. From these results the conclusion is drawn in § 7 that in order to bring about inhibition, the auxin coming from the paste needs to react or co-operate with some second factor which is supplied to the stem by leaves and cotyledons, and possibly by other members too. The results therefore tend to support the "indirect" explanation of inhibition (Laibach, 1933; Le Fanu, 1936; Snow, 1937), according to which the inhibiting influence originates indirectly from some reaction promoted by the auxin in the main stem.

5. Reasons are given in § 6 for concluding that upward inhibition of the main shoot by auxin paste is the same process as inhibition of lateral buds by auxin paste applied to the main stem, and the same also as natural correlative inhibition. Consequently the conclusions drawn from the experiments with auxin paste may be applied to correlative inhibition also.

6. Some further points are discussed at the ends of §§ 3, 4 and 5, and in § 7.

7. A theory of inhibition proposed by Van Overbeek (1938) is discussed in § 8.

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THE SYSTEMATIC POSITION OF THE FUCALES

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(With 5 figures in the text)

THE Fucales are probably the most widely distributed and the most abundant of all the brown algae. They have certainly reached the highest level of morphological and anatomical differentiation, yet their systematic position remains one of apparent isolation, and it has recently been claimed that they have been derived independently of all the other brown algae (Dekker, 1929).

The discovery of the microgametophyte of *Laminaria* by Sauvageau (1915) and Kylin (1916 *b*) focused attention upon the origin of this group and also lead to speculation as to some similar course of evolution for the Fucales. A primitive (probably heterosporous) type had zoospores germinating *in situ*, the enclosed gametophytes became suppressed leaving only the gametes, which are thus in a sense homologous with the zoospores. An approach to this condition was seen by Sauvageau (1915) in *Saccorhiza bulbosa*, where a few zoospores which had failed to escape germinated within the sporangium. Some such interpretation has been widely held (cp. Kylin, 1916 *a*, 1918; Lloyd Williams, 1921; Svedelius, 1927, 1929), but whilst the discovery of the life cycles of such types as *Asperococcus* or *Desmarestia* have strengthened the idea of relationship between Laminariales and Ectocarpales, no such links have been found between the Fucales and the lower Phaeosporae.

It is well known that meiosis occurs in the young unilocular sporangium¹ and also in the early stages of the oogonium and antheridium; but in their later development striking differences are found. In the latter, when nuclear multiplication is completed, delicate protoplasmic partitions form giving a number of antherozoid mother cells within each of which an antherozoid is developed (Fig. 1 *d*). In the oogonium of *Fucus vesiculosus* also evanescent wall formation has been described (Farmer & Williams, 1898). This septation recalls the plurilocular rather than the unilocular sporangium and according to Kylin (1933, 1937) entirely invalidates the comparison with the unilocular sporangium of the Phaeosporae. A further difficulty on which Kylin now lays much stress is the different ciliation of the zoospore and antherozoid. On both these grounds, Kylin now regards the Fucales as an isolated family originating from an unknown ancestor, and has recently expressed this view in the following emphatic words: "die Oogonien und die Antheridien der Fucaceen

¹ Cytological evidence of meiosis has been advanced for the genus *Chorda* (Kylin, 1918); for *Pterygophora* (McKay, 1933); for *Desmarestia* (Schreiber, 1932; Abe, 1938) and for *Eisenia arborea* (Hollenberg, 1936). Meiosis is also inferred for other genera in which the regular alternation of a sexless and sexual generation occurs. The cytological evidence advanced for *Pylaiella* (Knight, 1923), *Ectocarpus siliculosus* (Knight, 1929) and *Phleospora brachiata* (Mathias, 1935) makes it probable that the same rule holds for most if not all of the Ectocarpales.

lassen sich nicht von den unilokularen Sporangien ableiten; von einem anderen Gesichtspunkte; die Fucaceen haben sich *nicht aus den Phaeosporen entwickelt*" (Kylin, 1937, p. 28). Such a complete change of opinion by so eminent an algologist as Kylin must command attention, but this conception of the Fucales as a group unrelated to any known brown alga should not be accepted without further examination of all the available evidence from vegetative as well as reproductive structures. The following pages offer a brief review of known facts most of which have not been taken into account in the publications quoted above.

A. *Reproductive structures.* The antheridium arises on a branched filament very similar to the branched filaments bearing plurilocular sporangia in certain species of *Ectocarpus* (cp. *E. faroensis* Borg. or *E. monocarpus*¹). The development of the antheridium has been more closely studied in *Fucus* than in any other genus in the family; the earlier stages in *F. vesiculosus* (Yamanouchi, 1909) and *F. serratus* (Kylin, 1918) bear unmistakable resemblance to the Phaeosporean unilocular sporangium of corresponding age—for instance, *Ectocarpus* (Knight, 1929), *Phloeospora* (Mathias, 1935), or *Heterochordaria* (Abe, 1936). These resemblances include: the initial large central nucleus; meiosis at the first nuclear division; short pause after the second division and further simultaneous divisions until 32 nuclei are formed (Yamanouchi, 1909; Kylin, 1918).

The final differentiation of antherozoid and zoospore is very different. In the antheridium the protoplast forms finely granular partitions between the 32 nuclei: the latter divide again with the formation of further protoplasmic septa, well defined but delicate and entirely lacking in cellulose. Within each of the 64 antherozoid mother cells thus formed the antherozoid itself develops as shown by Guignard (1889). On the other hand, in the unilocular sporangium of higher as well as lower types, the zoospore is organized by the aggregation of plastids and plasma around each nucleus without any suggestion of partitions (Fig. 1 a, b). Finally, the protoplast is cleft into as many portions as there are nuclei, each piece rounding off to become a zoospore. The septation of the antheridium has been compared with that of the plurilocular sporangium² whether its products are sexual or asexual. In the plurilocular sporangium, however, the septa are firm cellulose walls produced successively after each nuclear division and usually enclosing rectangular compartments³ clearly visible after the escape of the zoids. The delicate protoplasmic septa of the antherozoid mother cells, on the other hand, break down completely at the release of the antherozoids. If the fucalean condition is due to the retention of a reduced gametophyte, the late formation of more delicate septa may be regarded as a vestigial character, the antheridium combining the diploid character of the unilocular with the septation peculiar to the plurilocular sporangium. A similar explanation can be applied to the oogonium of *Fucus vesiculosus* in which evanescent

¹ As figured in Oltmanns' *Morphologie und Biologie der Algen*, p. 13, 1922.

² Plurilocular sporangia may occur on the diploid or haploid generation and in either case the zoids may simply germinate to reproduce the parent thallus: ultimately, however, in most types, the haploid plurilocular sporangia produce gametes. According to Ueda (1930-2), in plurilocular sporangia which produce asexual zoospores, the septa disappear very early.

³ Occasionally, however, the segmentation is much less regular, as in *Ectocarpus terminalis* Kütz. (Kylin, 1937), and here the resemblance to the antheridium of *Fucus* is closer.

walls are formed (Farmer & Williams, 1898) and presumably also to those better developed walls in the oogonia of *Pelvetia*, *Xiphophora*, *Hormosira*, *Notheia* and *Durvillea* as to the fate of which little is known.

The second important feature stressed by Kylin is the difference in ciliation of the Phaeosporean and the Fucalean zoid. The significance of this lies in the assumption that each is constant within its own group. Such a constancy has,

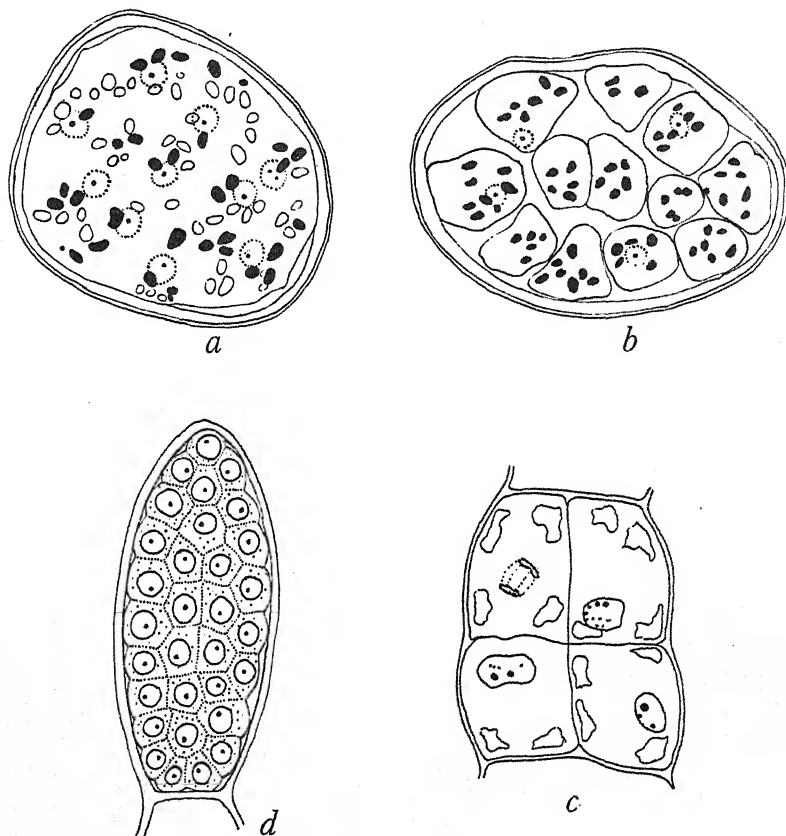


Fig. 1. Diagram showing late stages in development of Phaeosporean sporangium and of Fucalean antheridium. *a*, *b*, unilocular sporangium (after Higgins, 1931). *a*, before cleavage of cytoplasm: each nucleus is associated with plastids (shown black) and granular particles (in outline). *b*, after segmentation of cytoplasm. *c*, plurilocular sporangium of *Pylaiella littoralis* (after Knight, 1923) showing formation of thin cellulose partitions prior to organization of zoospores. *d*, antheridium of *Fucus vesiculosus* (after Kylin, 1916) just before development of antherozoids.

however, never been established (cf. Table I) and in the lower Phaeosporae, for example, it cannot be assumed that the asexual zoid and the gamete are alike in form and ciliation.

In all the examples of *asexual* zoids which have been recorded, the cilia diverge widely from their point of origin and in nearly all the anterior cilium appears to be the longer. However, if the published illustrations are to be trusted, it is clear that

Table I. Ciliation of zoospores and gametes

	Author	Magnification given	Ratio of lengths of cilia	
			Anterior	Posterior
A. Zoospores				
<i>Chaetopteris plumosa</i>	Kuckuck (1912 <i>a</i>)	× 1200	2.5	1.0
<i>Dictyosiphon foeniculaceus</i>	" "	× 1200	1.7	1.0
<i>Nemoderma tingitana</i>	" (1912 <i>b</i>)	× 1200	1.7	1.0
<i>Phloeospora brachiata</i>	Mathias (1935)	× 1000	1.4	1.0
<i>Zanardinia</i>	Yamanouchi (1913)	?	1.4	1.0
<i>Sphacelaria bipinnata</i>	Papenfuss (1934)	?	1.5	1.0
<i>Ectocarpus siliculosus</i>	Knight (1929) (1)	× 5000	1.8	1.0
"	" (2)	× 5000	2.4	1.0
<i>Eudesme virescens</i>	Parke (1933) (1)	× 1000	2.7	1.0
"	" (2)	× 1000	2.0	1.0
"	" (3)	× 1000	3.0	1.0
<i>Acrothrix novae-anglicae</i>	Taylor (1928) (1)	× 1250	3.6	1.0
"	" (2)	× 1250	1.0	3.0
<i>Mesogloia vermiculata</i>	Parke (1933)	× 1800	3.7	1.0
<i>Punctaria</i> .sp.*	Ueda (1930-2)	Measured	1.2	1.0
		5 × 7-8μ		
B. Gametes				
<i>Fucus vesiculosus</i>	Kylin (1920) (1)	—	1.0	1.4
"	" (2)	—	1.0	2.1
<i>Coccophora</i>	Tomita (1932)	—	1.5	1.0
<i>Phloeospora</i>	Mathias (1935) (1)	—	1.0	3.1
"	" (2)	—	1.0	2.3
"	" (3)	—	1.0	1.3
"	" (4)	—	2.0	1.0
<i>Punctaria</i>	Ueda (1930-2) (1)	—	1.0	1.8
"	" (2)	—	1.0	1.5
<i>Dictyota dichotoma</i>	Williams (1904)	Zeiss 3 mm. apochromat.†	2.8	1.0

* The anterior cilium is definitely the shorter in each of the five gametes figured by Ueda for *Punctaria*; but he describes them as "fast diesselbe Grosse und Organization" as the asexual zoids. It is stated that *Scytosiphon* and *Endarachne* behaved in the same way as *Punctaria*, but no figure of their zoids is given.

† Posterior cilium usually not to be discerned.

the relative lengths of the two cilia are by no means constant even in the same type. The numbers in Table I were obtained by careful measurement of the cilia in favourable examples of zoospores drawn under high magnification by various authors (cp. Fig. 2). Even allowing for difficulties of technique and observation, the range is so great as to suggest that the relative lengths of the two cilia is a variable (probably adaptive) feature rather than constant throughout the group. Moreover, the zoospores of *Acrothrix* (Fig. 2) are apparently dissimilar, two having the shorter cilium in the anterior position (as in the antherozoid of the Fucales) and one the reverse. Both these genera belong to the higher Ectocarpales¹ (to the Chordariaceae and Striariaceae respectively), but most of the genera in these and allied families have not yet been critically examined and there is good ground for believing that the whole range of variation of the cilia of the zoospore is still unknown.

¹ For the purposes of this article, the "higher Ectocarpales" are regarded as including the Encoeliaceae, Chordariaceae, Striariaceae, Myriotrichaceae and Mesogloiaaceae.

It is generally believed that the *gametes* of the Phaeosporae have the longer cilium in the anterior position, and this is certainly true of the Sphacelariaceae and Cutleriaceae (Yamanouchi, 1913; Kuckuck, 1912; Papenfuss, 1934). In the Laminariales the male gametes have rarely been seen; the only example known to the writer in which one has been figured is that of *Macrocystis* (Levyns, 1933) where the orientation of the cilia cannot be judged owing to the form (or aspect) of the gamete

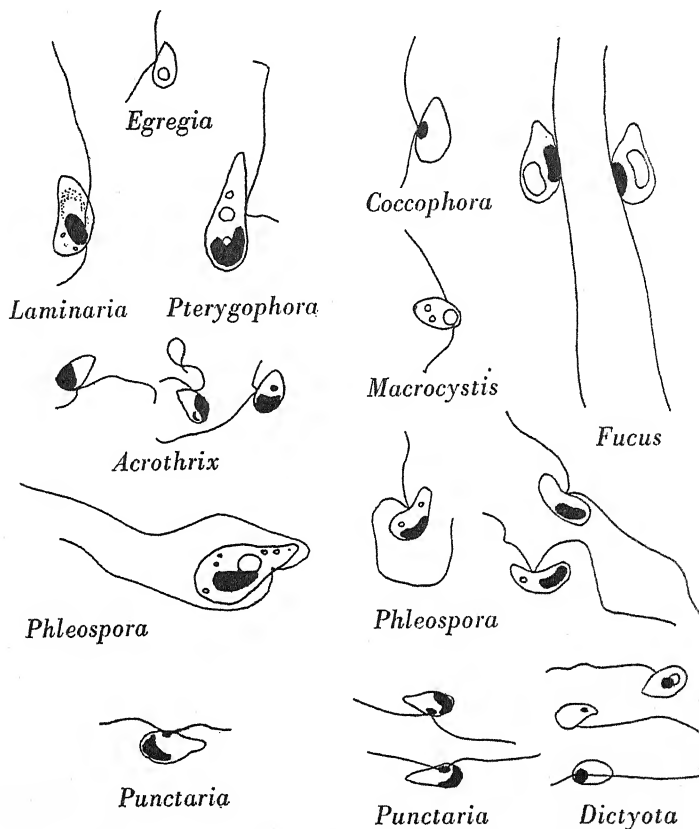


Fig. 2. Zoospores and gametes of Phaeophyceae. Zoospores: *Egregia* (Myers, 1927-9); *Laminaria saccharina* (L.) Lamour (Kuckuck, 1912 a); *Pterygophora* (McKay, 1933); *Acrothrix* (Randolph Taylor, 1928); *Phleospora* (Mathias, 1935); *Punctaria* (Ueda, 1930-2). Gametes: *Fucus vesiculosus* (Kylin, 1918); *Coccophora* (Tomita, 1932); *Macrocystis* (Levyns, 1933); *Punctaria* (Ueda, 1930-2).

(cp. Fig. 2). The gametes of the Ectocarpales are still but little known, but recently in three instances they have been depicted with the anterior cilium shorter, as in the genus *Fucus* (cp. Table I, and Fig. 2). In one of these, *Phleospora*, four of the five (unfused but undoubted) gametes have the short cilium anterior (cp. Fig. 2), the fifth has the short cilium in the posterior position.¹ There can be little doubt that when the life histories of the higher Ectocarpales are more fully known and closer attention paid to this question, other examples of the kind will also be found.

¹ The author has kindly informed me that the observations were made on living material and that the relative lengths of the cilia did not come under special scrutiny. However, taking into consideration the marked inequality of the cilia, it seems most likely that these are truly representative.

The character of the Fucal antherozoid has been mostly deduced from that of *Fucus*, which was closely studied by Guignard, Kylin and others. It is impossible to find detailed representations of the antherozooids of other genera, but in the recently described *Coccophora Langsdorffii* (Turn.), a Japanese type probably closely allied to *Sargassum*, the antherozooids figured by Kogoro Tomita (1932) have the longer cilium in the *anterior* position, although no attention is drawn to this fact in the text. It is thus extremely doubtful whether there is any constancy in the ciliation of the *Fucales*¹ and there is certainly considerable variability within the *Phaeosporeae*. It is of particular interest that the zooids of *Acrothrix* can be found with the anterior cilium short like that of *Fucus*, since there are also certain similarities in their vegetative structure (cp. p. 235).

VEGETATIVE STRUCTURE

Since the *Fucales* cannot be regarded as completely isolated on the grounds proposed by Kylin, it may be well to seek for any further signs of affinity with the lower *Phaeosporeae*, and especially with the *Ectocarpales*.

One of the most striking differences between these groups lies in the apical growth of the one and the intercalary (trichothallic) growth of the other. This difference has been largely explained by the brilliant work of Nienburg (1931) on the sporeling of *Fucus vesiculosus* published shortly before his death. In the main, the course of development in the very young sporeling was found to conform to the description given by Oltmanns (1889); but Nienburg was able to grow his sporelings in culture for a longer time than Oltmanns, in spite of practical difficulties,² and by sectioning these older sporelings it was found that a small group of apical hairs formed at the growing point, each with a basal region of active cell division. At a slightly later stage, one or more of these hairs died off nearly to the base and the lowest cell of one of the hairs (often the most central) finally give rise to the apical cell of the future thallus (cp. Fig. 3 e-h). Similar stages were also recognized in sporelings of appropriate age collected from the shore. Evanescent apical hairs are recorded for other genera of the *Fucales* (*Pelvetia*, Oltmanns, 1889; *Xiphophora*, Heine, 1932); they are also characteristic of the higher families of the *Ectocarpales* and occur, for example, in sporelings of *Adenocystis* (Skottsberg, see Oltmanns, 1922) and in *Acrothrix* (R. Taylor, 1928; Parke, 1933) where, at least in the Californian species described by Randolph Taylor (1928), the apical hair falls off in the older thallus, leaving, however, one of its lowermost cells to segment after the manner of a true apical cell. The apical hair itself, though arising relatively late in the life of the sporeling, strongly suggests affinity between the *Fucales* and the lower *Phaeosporeae*: but it was found by Nienburg that in *Fucus vesiculosus* adventitious shoots arising after injury are also initiated by the formation from the inner tissues

¹ It may be noted in passing that the antherozooids of the *Fucaceae* of the southern hemisphere are practically unknown from this point of view and may be expected to yield interesting results if examined by appropriate methods.

² In culture solution the sporelings were badly overgrown with diatoms: they fared better in sterilized sea water only, although then deprived of nutriment: the apical hairs were found in sporelings about 6 weeks old.

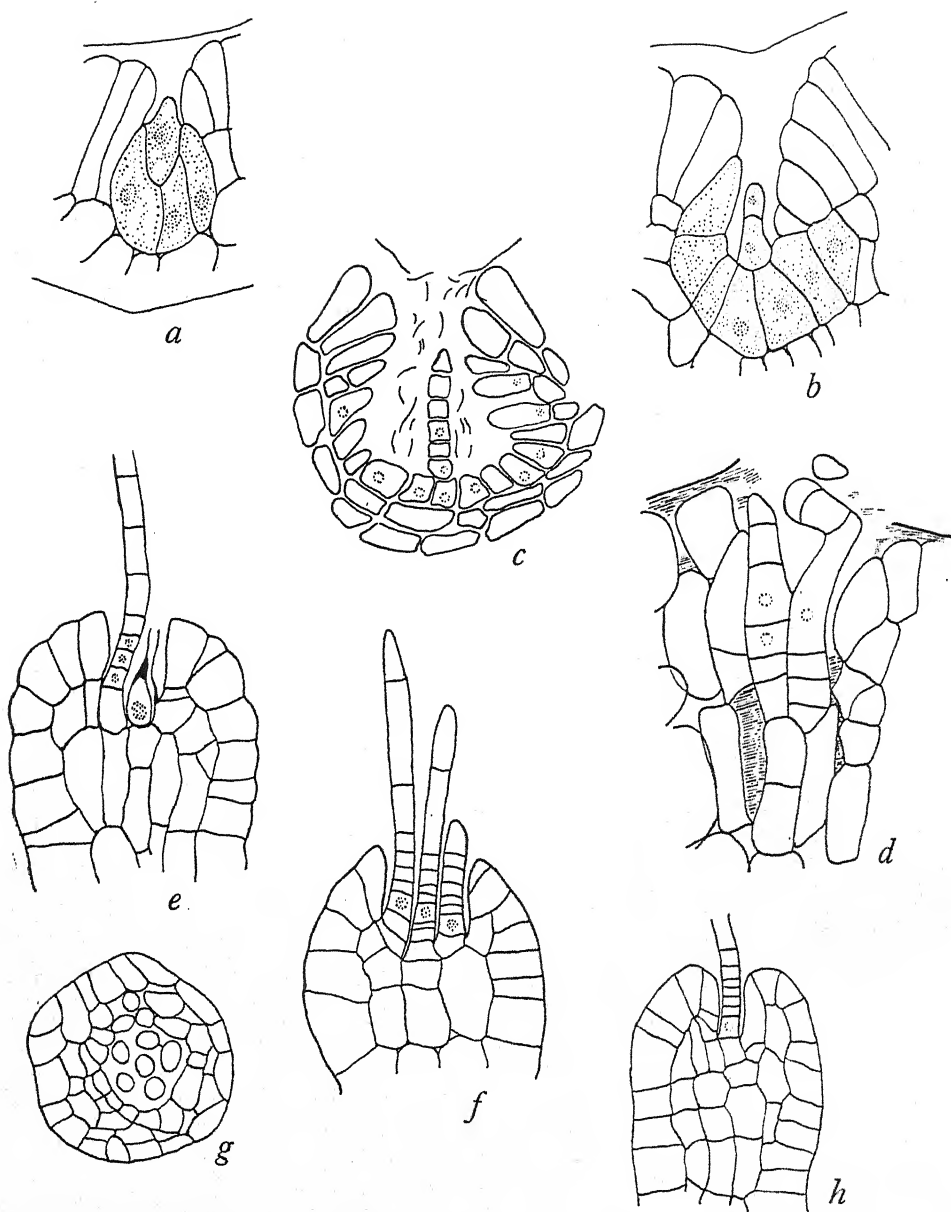


Fig. 3. *a-c*, early stage in development of conceptacle. *a, b*, *Halidrys siliquosa* (after Nienburg, 1913). *c*, *Halidrys dioica* (after Doubt, 1932). *d*, regeneration in *Fucus vesiculosus* (after Nienburg, 1931). *e-h*, apical hairs at apex of sporangia of *F. vesiculosus* showing origin of apical cell. *e, f*, several apical hairs; *h*, solitary apical hair of which lowest cell (shown with nucleus) will give rise to the four-sided apical cell; *g*, transverse section of sporangium at level of base of hairs: a centrally placed hair will lose its upper cells (as in *e*) and the remaining lowest cell will give rise to the apical cell. (After Nienburg, 1931.) Nienburg believed that the apical cell was formed by inclined walls within the special hair cell, but this was *observed* only in connection with regeneration.

(near the wound) of hairs with a basal region of cell division. Later these hairs die away, and from the base of one of them an apical cell is organized, either three-sided (temporarily) or four-sided, from which the reparatory tissues are segmented (Fig. 3 d).

THE CONCEPTACLE AND HAIR PITS

The origin of the conceptacle has been traced in most species to a superficial cell very near the growing point. This cell becomes depressed slightly below the surface owing to the more active growth of the cells around it. It then divides by a wall, transverse (*Himanthalia*, *Halidrys*) or curved (*Fucus*, *Cystoseira* and others) and the lower cell subsequently divides by delicate vertical walls to form a little group of cells with very rich contents, but which are easily overlooked in the early stages owing to the difficulty with which their walls take up any stain (Fig. 3 a, b, c).

In *Himanthalia* (Nienburg, 1913) and *Halidrys dioica* (Doubt, 1928), the upper segment of the initial cell forms a long multicellular hair with typical basal growth; the lower segment divides by vertical walls and forms the greater part or possibly the whole of the inner lining of the conceptacle from which the paraphyses and gametangia ultimately arise. In *Halidrys siliquosa* and *Cystoseira* there is little segmentation, the primary hair degenerating after one or two divisions (Nienburg, 1913), while in *Fucus*, *Bifurcaria* and *Sargassum* no further divisions take place, but the upper cell becomes elongated, pointing upwards like a short tongue towards the narrow osteole and degenerating very early, while the lower cell divides to form the layer of cells lining the floor of the conceptacle.¹ It can hardly be doubted that the initial cell of the conceptacle with its peculiar behaviour is a link with the lower filamentous brown algae. According to Moore (1928) the conceptacle of *Pelvetia fastigiata* is initiated by a group of hairs which behave in a similar manner. The latter seems to be unique among the Fucales at present known and is reminiscent of the hair pits or cryptostomata so commonly found on the sterile thallus in this group. These hair pits also arise from the overarching of a small group of cells near the growing point. From the base of the depression arise a number of long nearly colourless ectocarpoid hairs.² There is a striking resemblance between the fucal cryptostomata filled with projecting hairs and those of *Chnoospora* (Barton, 1898 b; Levring, 1938), *Colpomenia* or *Leathesia*, a resemblance which again suggests affinity.

There remains to be considered the general organization and structure of the thallus in the Fucales. In spite of much diversity of form and habitat, certain common structural features are clearly marked. These are:

(1) The apical growth (this we have seen can be referred to an intercalary origin).

¹ In *Pelvetia canaliculata* and in *Ascophyllum*, the lower half of the initial cell divides, forming irregular projections into the cavity formed by the active development of adjoining tissues. The further fate of these projections is in doubt.

² The function of these hairs must be a matter of speculation; in some *Fucus serratus* kept by the writer for about six weeks in a rather dimly lighted north window, the hairs grew to a length of several centimetres, covering the thallus with a cobweb-like growth. These hairs were unbranched and faintly pigmented.

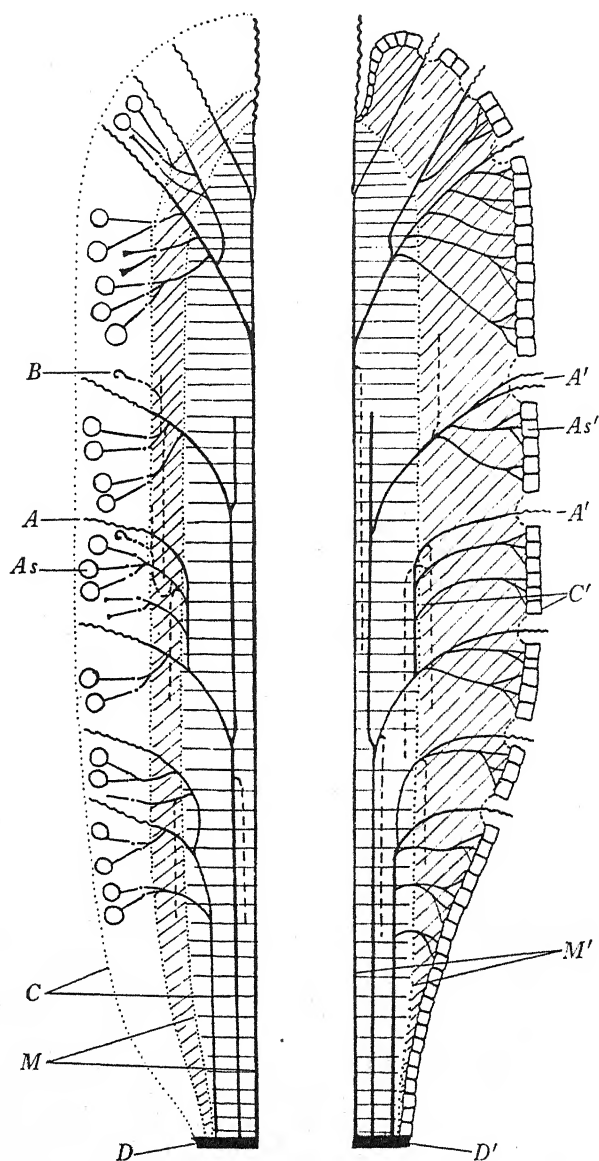


Fig. 4. Diagram illustrating probable origin of fucoid thallus. Left, "multiple strand" filamentous thallus of Mesogloioiaceae (after Parke, 1933, Text-fig. 2). Right, the same, compacted to form a parenchymatous thallus potentially that of the Fucales. *A*, colourless hair. *A'*, hairs in depression (as in cryptostomata). *As*, primary assimilators. *As'*, superficial layer of assimilating cells. *B*, secondary assimilator. *C*, *C'*, cortex, outer assimilating or storage, inner, storage, increasing by down-growing filaments. *M*, *M'*, medullary tissue. *D*, original disk. *D'* attaching disk.

(2) The primary structure of medullary filaments surrounded by "cortical" cells, with no very clear demarcation between the inner storing region and the outer assimilating layers.

(3) The secondary thickening brought about by (a) intercalation of hypha-like outgrowths chiefly from the inner cortical cells; (b) by periclinal and anticlinal divisions in the cells of the outer layers of the cortex of old stipes of *Fucus* and presumably also of other genera capable of long continued growth.

The medullary filaments are an important and characteristic feature of the fucoid thallus. They are differentiated in the very young sporeling (cp. Fig. 5 b) and are probably reinforced by the products of division of the apical cell when this is established. They may be seen clearly near the apex of the thallus of such genera as have been examined in detail (*Fucus*, *Ascophyllum* and others, cp. Fig. 5 d, e). In this position they are cylindrical cells about 3-6 times as long as broad, with mucilaginous vertical and delicate cellulose transverse walls. These cells retain their living contents, but appear not to undergo any further division. In some plants they remain short and the firm adherence of the protoplast to the transverse walls in *Bifurcaria* (Fig. 5 d, e), *Ascophyllum* and *Fucus* suggests an approach to the sieve tube, although "slime strings" have not been demonstrated. In *Marginariella*,¹ the sieve tube appearance is greatly strengthened by the presence of large callus pads on the transverse and lateral walls (Fig. 5 j, k) hitherto unrecorded for the Fucales. In all these, the conducting function is clearly indicated. In rapidly growing thalli, such as *Himanthalia*, the cells of the filaments become greatly stretched, the length being from 14-30 times the diameter.² Those in the centre become twisted on themselves (Fig. 5 h) or on each other (Fig. 5 i). Towards the base of the thallus (of surf plants especially) the central filaments are dragged apart by the numerous ingrowing lateral secondary filaments, and their lateral pit connexions are pulled out on short arms (Fig. 5 f); this displacement is increased by mechanical effects of wave action, and the filaments of old stipes are not easy to trace, although with care they can be teased out from the softened tissues. Some of the filaments, whether primary or secondary, have much thickened walls and appear to have also a mechanical function, giving great tensile strength and flexibility (cp. Hansteen, 1892; Delf, 1932).

Near the base of the thallus, the medullary filaments put out laterals which grow steeply downwards, branch and finally intertwine together forming the attaching disk. They become septate and at the periphery of the disk it is clear that the cell division is in the apical region,³ recalling the disk filaments of the Mesogloiaceae (Parke, 1933) or, more remotely, the apical growth of the basal filaments of the heterotrichous genus *Ectocarpus*.

In plants of relatively sheltered habit, as in deep pools (*Bifurcaria*, *Cystoseira*, *Halidrys*), the secondary medullary filaments are rare or altogether absent. The cells of the primary medullary filaments are short, straight and firmly cemented

¹ Unpublished observations on *Marginariella Urvilleana* made for the writer by Dr M. T. Martin.

² Ascertained by E. M. Rees from measurements of dissected filaments.

³ Compare Hansteen (1892), Wille (1910), and Rees (1933).

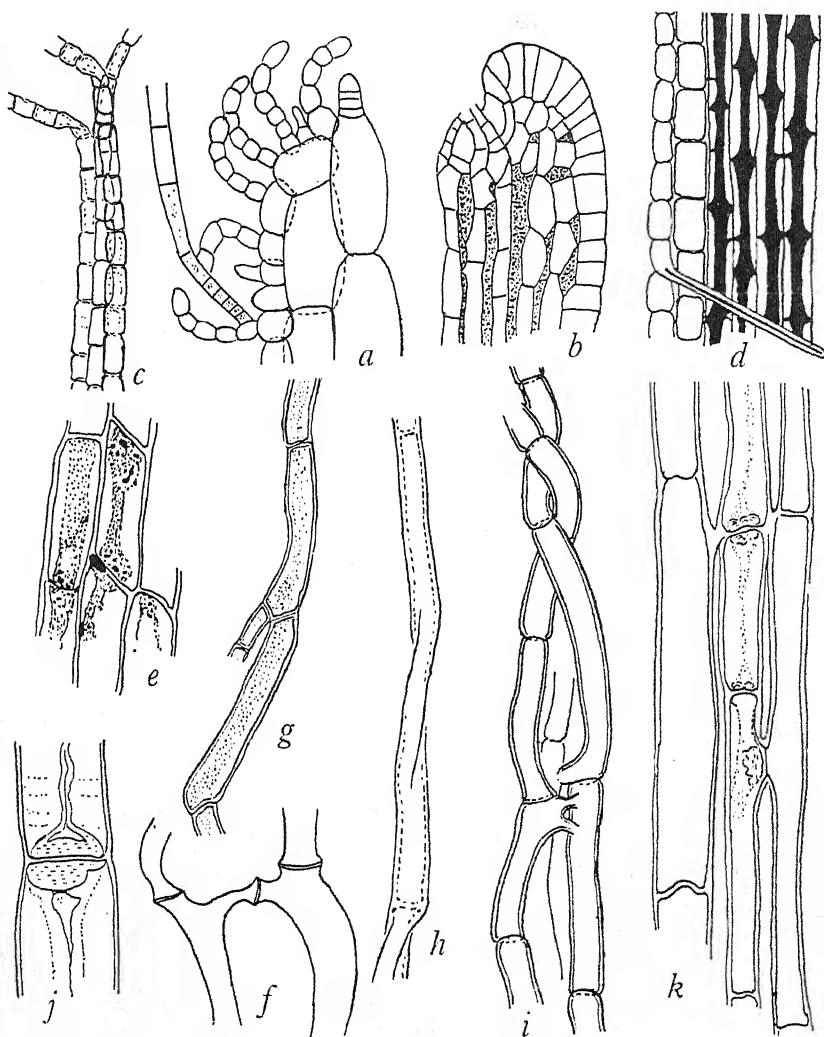


Fig. 5. Structural features of the Fucales and Mesogloiaceae. *a*, part of longitudinal section of apex of thallus of *Acrothrix*, after Oltmanns (1922). *b*, part of longitudinal section of apex of sporangium of *Fucus*, after Oltmanns (1922). *c*, typical central filaments from the thallus of *Eudesme*, after Parke (1933). *d*, part of longitudinal section of young thallus *Ascophyllum*, near apex; after Hick (1885), showing medullary filaments and two inner layers of cortical cells, one of which is giving off a young secondary filament. *e*, medullary filaments of *Bifurcaria* in longitudinal section, after Rees (1933). *f*, *g*, *Fucus serratus*, after Wille (1885). *f*, central filaments forced apart by secondaries (not shown); a lateral pit connexion pulled from its original position by the extension of the walls of both cells. *g*, central filaments with down-growing secondary filaments (cp. Parke, 1933, Fig. 2). *h*, *i*, *Himanthalia* (from unpublished drawings by E. M. Rees). *h*, single twisted cell from a central filament dissected from the medulla of lower part of an old thallus. *i*, two filaments intertwined, dissected from the medulla of a young thallus. *j*, *k*, *Marginariella Urvilleana* (Rich.) Tandy. central filaments from fertile region of thallus; from a drawing by Dr M. T. Martin (unpublished). *j*, callus pad on transverse wall. *k*, sieve-tube like cells with callus pads.

together (Fig. 5 e), so that in section the appearance of a parenchymatous tissue is obtained. In surf plants, on the other hand, the primary and secondary filaments are crowded and interwoven, so that in section there is a confused appearance, quite unlike that of the parenchyma of the higher plants (cp. Oltmanns, 1922, Fig. 453). The differences between the parenchymatous and the surf types may well be considered adaptive, but no explanation of their common fundamental features appears to have been put forward. In the opinion of the writer, such an explanation may be found from a consideration of the structure of the sporophyte of the Mesogloiaceae and related types.

According to Parke (1933) the ground plan of the young thallus of *Eudesme*, like that of the other Mesogloiaceae, is that of a central medulla formed of multiple strands or filaments surrounded by a "cortex" of the aggregated laterals from the central filaments. Both central filaments and laterals give rise to secondary downward-growing hypha-like filaments, the intrusion of which adds to the thickness of the thallus. The latter is soft and mucilaginous in *Eudesme*, so that "if the tip of a plant be taken and pressed gently between two glass slides, the coherence of the filaments is so slight that they readily separate fanwise into individual strands each having the appearance of an *Ectocarpus* provided with tufts of lateral branches and colourless hairs". In *Castagnea*, however, the longitudinal rows of filaments are more firmly cemented together and will not separate from one another even with considerable pressure; so that a somewhat parenchymatous character is attained. The secondary hypha-like filaments exhibit *apical* growth; if they reach the disk they interweave with similar filaments and form a more or less extensive hapteron. The differentiation thus briefly described has an obvious resemblance to Hansteen's classic description of *Fucus vesiculosus* (1892), where the principal categories of tissues were respectively distinguished as assimilating, cortical and medullary.

In *Acrothrix*, as described by Parke (1933), there is a similar but simpler organization, a single central filament ending in the characteristic ectocarpoid hair. In the species of *Acrothrix* described by Randolph Taylor (1928) the terminal hair was only visible in the young thallus, falling off with age just above the base where the actively dividing cells were placed. The latter appeared to retain their meristematic function and to bring about continued growth in length. We thus have an approximation to the apical growth of the fucoid thallus, initiated by the disappearance of an ectocarpoid hair and by the increased activity of one of its meristematic cells.

It is thus not difficult to derive a fucoid thallus from a multiseriate type like *Eudesme*, in which the terminal part of one of the central filaments gives rise to an apical cell as in the young thallus of *Acrothrix*, and as in the sporeling of *Fucus vesiculosus* (p. 229). The change from the almost parallel course of the secondary filaments of the Mesogloiaceae (Parke, 1933; Figs 2, 4, this article) to the laterally directed or obliquely interwoven hyphal filaments of the surf-loving genera of the Fucales may well have been induced by the stresses and strains to which they are constantly exposed which must frequently involve local internal displacements. The primary disk of the Mesogloiaceae finds its counterpart in the characteristic

discoid hapteron of the Fucales, both being formed of interwoven apically growing septate filaments (p. 233, text, and footnote 3; and Parke, 1933, Pl. VIII, fig. 49).

Not only can the apical growth and the general structure of the thallus of the Fucales be explained intelligibly along these lines, but the development of the conceptacles can be compared with that of the sporangia from the assimilators of the Mesogloiaceae and its allies, or still more, with that of sporangia which arise in connection with assimilators in shallow sori around hair pits in *Leathesia* or *Soranthera* (Fig. 4). It is not necessary to develop the comparison at this stage, but enough has been said to support the view that the Fucales are far from having an isolated position. They show actual points of affinity with the Mesogloiaceae on the one hand and with the Encoeliaceae on the other, both families being typical of the higher Ectocarpales. These signs of affinity may be discerned in:

- (1) The general organization of the whole thallus structure.
- (2) The presence of hairs with basal growth in the cryptostomata and at the initiation of the conceptacles.
- (3) The short-lived ectocarpoid hairs at the growing point of the sporeling preceding the formation of the apical cell.
- (4) The formation of hair-like outgrowths at regeneration from a wound.
- (5) The resemblances between the earlier developmental stages of antheridia and oogonia and unilocular sporangia.
- (6) The vestigial septation of antheridia and some oogonia in the later stages of development and its possible relation to the septation of haploid plurilocular sporangia.
- (7) The common features of antherozoid and Phaeosporean zoid, especially the widely divergent and laterally placed cilia, the differences in length of cilia being secondary and probably adaptive.
- (8) It must be concluded, therefore, on all these grounds that the Fucales, like the Laminariales, represent the climax of a line of development from a filamentous ancestor having an alternating summer macrosporophyte and winter gametophyte as now seen in certain genera of the Ectocarpales. It follows that the summer sporophyte must have acquired the perennial habit and that the gametophyte must have suffered extreme reduction. An increasing vigour in the diploid generation leading to longer life and greater differentiation may be correlated with the number of chromosomes which is markedly greater in the Fucales than in the lower types;¹ but what may be said as to the almost total disappearance of the gametophyte?

In the Mesogloiaceae and the Encoeliaceae the gametophyte is ectocarpoid, consisting of creeping septate threads, pigmented and bearing typical plurilocular sporangia from which gametes issue.² It is clearly dependent on a *submerged* existence and is thus normally surrounded by dim bluish light of short duration at

¹ In *Ectocarpus*, *Myriotrichia* and *Punctaria* the haploid number is 8, in the Sphacelariaceae 16 (see Knight, 1929); in *Heterochordaria* there are 20 haploid chromosomes (Abe, 1936); and in the Fucaceae, 32 have been found for the following: *F. vesiculosus* (Yamanouchi, 1909), *Sargassum* spp. (Tahara & Shimotomai, 1926; Okabe, 1929; Abe, 1935), *Coccophora* (Tahara, 1929; Tomita, 1932), and *Cystophyllum* (Shimotomai, 1928).

² These types are homosporous, and just where heterospory entered into the heritage of the Fucales is at present beyond conjecture.

a temperature lower but more equable than that of the summer sporophyte. It has already been seen that the sporophyte¹ of many of the Fucales is adapted for *emergent* life. This emergence must have greatly jeopardized the escape and subsequent fate of the zoospores of the hypothetical prototype, but their germination within the sporangium would not necessarily have been prevented, especially if the latter were grouped in soral depressions or rudimentary conceptacles. By analogy with the gametophyte of the Laminariales (Harries, 1932), we may suppose that the fucoid microgametophyte also required a low temperature and illumination for active sexual reproduction and that the purely vegetative part was unable to develop partly owing to inadequate illumination within the conceptacle, partly owing to the variable and often higher temperature during exposure.

If this theory be accepted, the fact that intertidal genera of the Fucales are mostly fertile in the colder months finds explanation, as well as their geographical predominance in the colder zones. In warmer waters these give way to submerged types (*Cystoseira*, *Sargassum*, etc.), and here the frequent extrusion of the contents of the oogonium in the 8-nucleate stage (cp. Nienburg, 1912; Tahara, 1911-13; Delf, 1937, 1939) is, in effect, the liberation of a small haploid gametophyte from which a solitary oosphere will be formed by degeneration of all but one of the nuclei.

The life history of the Fucales, though specialized and contracted, can thus be brought into relation with that of the lower filamentous types such as are now becoming well known in the higher Ectocarpales. The greater complexity of the thallus and the reduction of the gametophyte can be explained with reference to the emergent habit. The sporophyte became adapted to the amphibious existence of the surf plant, or (probably secondarily) to greater submergence. The gametophyte, on the other hand, more sensitive than the sporophyte to the changes in illumination, temperature and concentration of sea water inevitable in an emergent type, lost all its vegetative features and only survived as a short phase represented by a few nuclear divisions within the oogonium while still protected by the conceptacle of the parent plant. In this connexion the mucilage developed by the conceptacle played an important part, not only in drought resistance during exposure, but in various mechanisms for the extrusion of the gametes so that fertilization is still external and aquatic as in more primitive types.

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¹ The diploid generation of the Fucales is regarded by the writer as essentially sporophytic; others prefer to describe it as a "diploid gametophyte".

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STUDIES IN THE EFFECTS OF PROLONGED ROTATION OF PLANTS ON A HORIZONTAL KLINOSTAT

II. ANATOMICAL STRUCTURE

By E. D. BRAIN, F.L.S.

(With 5 figures in the text)

INTRODUCTION

MORPHOLOGICAL changes, produced by growth on a klinostat, were noted by Czapek (1898) in *Marchantia* sporelings ("Brutkörper"), which lost their dorsiventrality and resembled tube-like thallus leaves.

Helen A. Dorety (1908) made the discovery that embryos of *Ceratosamia*, which normally have one cotyledon, developed two on the klinostat. Hermann Bücher (1906) described variations found in the tissues of stems which were laid horizontal and prevented from bending geotropically. Repeating one of his experiments with seedlings of *Cucurbita pepo* the writer found that thickened walls and smaller lumina were produced in the collenchyma of the upper side of horizontally placed hypocotyls and thinner walls and larger lumina on the lower side. It was also found that seedlings of *C. pepo* grown entirely on a horizontal klinostat had thinner walled collenchyma than upright ones, and that in *Lupinus polyphyllus*, when grown on a klinostat, the shape of the cross-section of the hypocotyl and the shape of the cortical cells were altered (Brain, 1926).

This paper deals with an anatomical investigation of *Narcissus pseudo-narcissus* flowers and leaves, *Asplenium bulbiferum* fronds and *Lupinus albus* seedlings grown for prolonged periods on a horizontal klinostat. The effect of prolonged rotation on a klinostat on their growth rate has been described previously (Brain, 1935). An exhaustive study of each species has not been attempted, but a comparison of general structure has been made with a view to ascertaining in what directions growth on a klinostat may affect the plant morphologically.

The distribution of statolith tissue and the shape and size of cells have been studied in detail.

METHODS

Plants were grown in a greenhouse at a temperature of about 20° C. and 75-90 % humidity. The klinostats rotated once an hour, and all plants during experiments were kept equally moist.

Fronds of *Asplenium bulbiferum*, flowers and leaves of *Narcissus pseudo-narcissus* and seedlings of *Lupinus albus* were fixed in 70 % alcohol. To enable examination of statolith tissue they were laid horizontal, previous to fixation, for a period longer than their gravitational presentation time, when fallen starch grains are found on

the lower walls of the cells. Sections were cut by hand and examined in iodine and glycerine.

RESULTS

Narcissus pseudo-narcissus. As is well known, flowers of this plant, when grown entirely on a revolving horizontal klinostat, remain in a straight line with the peduncle and do not show the normal bend of the receptacle as the bud develops. Examination of transverse sections cut at different levels of the receptacle at different stages of development show that there is a progressive development of statolith tissue as the flower opens which may be summarized in the following stages:

(1) *Upright bud stage*. Either two or four statocytes occur round each vascular bundle of the peduncle. Statoliths are found in the starch sheaths round the outer bundles of the peduncle at the base of the bud, and scattered starch in large quantities in the ground parenchyma. At the separation of the bud sheath the starch disappears from the sheath bundles and statoliths appear round the outer bundles of the receptacle. Higher up the receptacle and in the ovary wall a small quantity of scattered starch is found round the bundles.

(2) *Bud bending*. The stem is as in stage (1). At the base of the bud statoliths occur in the starch sheaths of the outer bundles. At the base of the bud sheath statoliths are found round the bundles of the sheath and the outer bundles of the receptacle. Higher up, above the separation of the sheath, the starch disappears from the sheath and statoliths are found round all bundles of the receptacle. At the base of the ovary there are quantities of statocytes round all the bundles, in the ovary wall and the placenta there are several statocytes to all bundles, and there is scattered starch at the base of the style. A few statocytes are found round the bundles at the base of the perianth, and two statocytes to each bundle at the level of the separation of the stamens.

(3) *Sheath burst, bud bent 90°*. Distribution of statocytes is similar to the previous stage up to the top of the ovary. At the base of the perianth there are statocytes all round the bundles and several statocytes to each stamen bundle. There is scattered starch at the base of the style. Above the separation of the stamens there are fewer statocytes in the perianth.

(4) *Flower open*. The stem is the same as in previous stages. Half-way between the base of the sheath and the ovary a single row of statocytes occurs round each bundle of the receptacle. There is a single row of statocytes round each of the bundles of the ovary wall and the placenta and at the base of the perianth. There is scattered starch at the base of the style, and above the level of the separation of the stamens there are four statocytes to each bundle in the perianth.

(5) *Flower withering*. No statoliths remain and all starch disappears except from the starch sheaths round the bundles of the receptacle below the ovary.

(6) *Flower withered*. The statoliths become embedded. Some scattered starch persists in the placenta.

It is evident that the maximum development of statolith tissue coincides with the bending of the receptacle as the flower develops. This has been illustrated diagrammatically in Fig. 1.

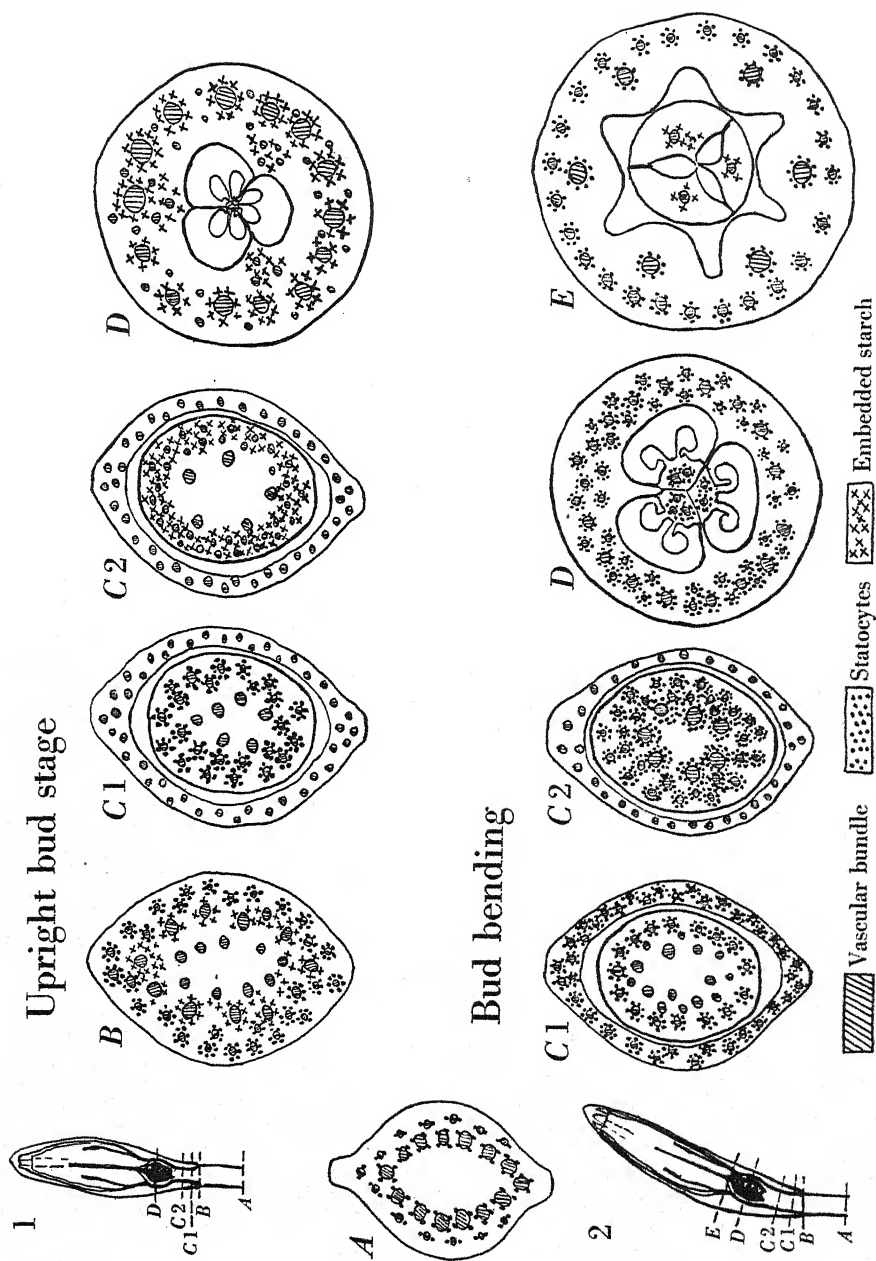


Fig. 1. *Narcissus pseudo-narcissus*. Diagrams to show development of statolith tissue in flower buds. 1. Upright bud stage. Transverse sections through planes A-D respectively. 2. Bud bending. Transverse sections through planes A-E respectively. A and B are similar for both stages.

For comparison of the structure of flowers grown upright and on the klinostat numerous sections have been examined for corresponding stages throughout development. No difference in the development of the statolith tissue, either in time or space, has been found between flowers grown upright and on the klinostat. The stages described above hold good for both series, and the quantity of statocytes and the quantity and size of the statoliths appear to be similar.

Comparison of the size and shape of statocytes shows variations in both series.

There is no difference apparent in the arrangement of the vascular tissue when plants are grown on the klinostat, but there are differences of size and shape in the cells of the outer tissues of the peduncle and receptacle.

In the *peduncle* there is no difference in the thickness of the epidermal wall, but the average radial diameter of epidermal cells, as seen in transverse section, is shorter on the klinostat. Palisade cells are bigger and statocytes slightly smaller and more nearly iso-diametric in shape on the klinostat than in upright plants.

In the *receptacle* the outer epidermal wall is the same thickness and no appreciable difference is shown in the size and shape of cells for the upright bud stage of development. At the stage when the bud bends normally, differences occur in the outer layers of cells. Fig. 2 is an attempt to show diagrammatically the changes which occur in the shape of the outer layers of cells in the receptacle when the flower is bent. The averages for measurements of cells are recorded in Table I and their shape is expressed as the ratio of their two diameters. The terms radial and tangential diameters are used for the axes of the cells lying in the radial and tangential planes of the section.

From *B-C* (Fig. 2) the outline of the transverse section is elliptical and the bend occurs in the plane of the shorter diameter. At *B*, in transverse section, cells of the epidermis are rectangular and those of the palisade layer iso-diametric in shape. At *C*, cells of the outer layers from *a-a'*, *b-b'*, are stretched in a radial plane and compressed tangentially, whereas at the sides of the receptacle *a-b* and *a'-b'* they are compressed both radially and tangentially. Longitudinal sections in the plane of the bend show that the cells of the concave part of the receptacle are compressed to more than half the length of those of the upper convex side. On the klinostat the bend does not occur in the receptacle. The transverse section from *B-C* (Fig. 2) is elliptical. At *B* the epidermal cells are slightly longer in the radial plane and have shorter tangential diameters than in upright flowers. At *C*, from *a-a'* and *b-b'*, which corresponds with the convex and concave sides of upright flowers, the cells are compressed radially and tangentially; but from *a-b* and *a'-b'* cells are compressed radially and stretched tangentially. In longitudinal section of the flowers grown on the klinostat, cells are longer, and slightly narrower radially than on the upper side of upright flowers. It therefore appears that on the klinostat the cells which correspond to the upper and lower ones in upright flowers remain unaltered in shape, but are compressed, and the cells at the sides are altered in the epidermis by becoming more iso-diametric in shape in the palisade layer by stretching in a tangential plane. Cell walls are thinner in the klinostat flowers, especially from *a-a'* and *b-b'*.

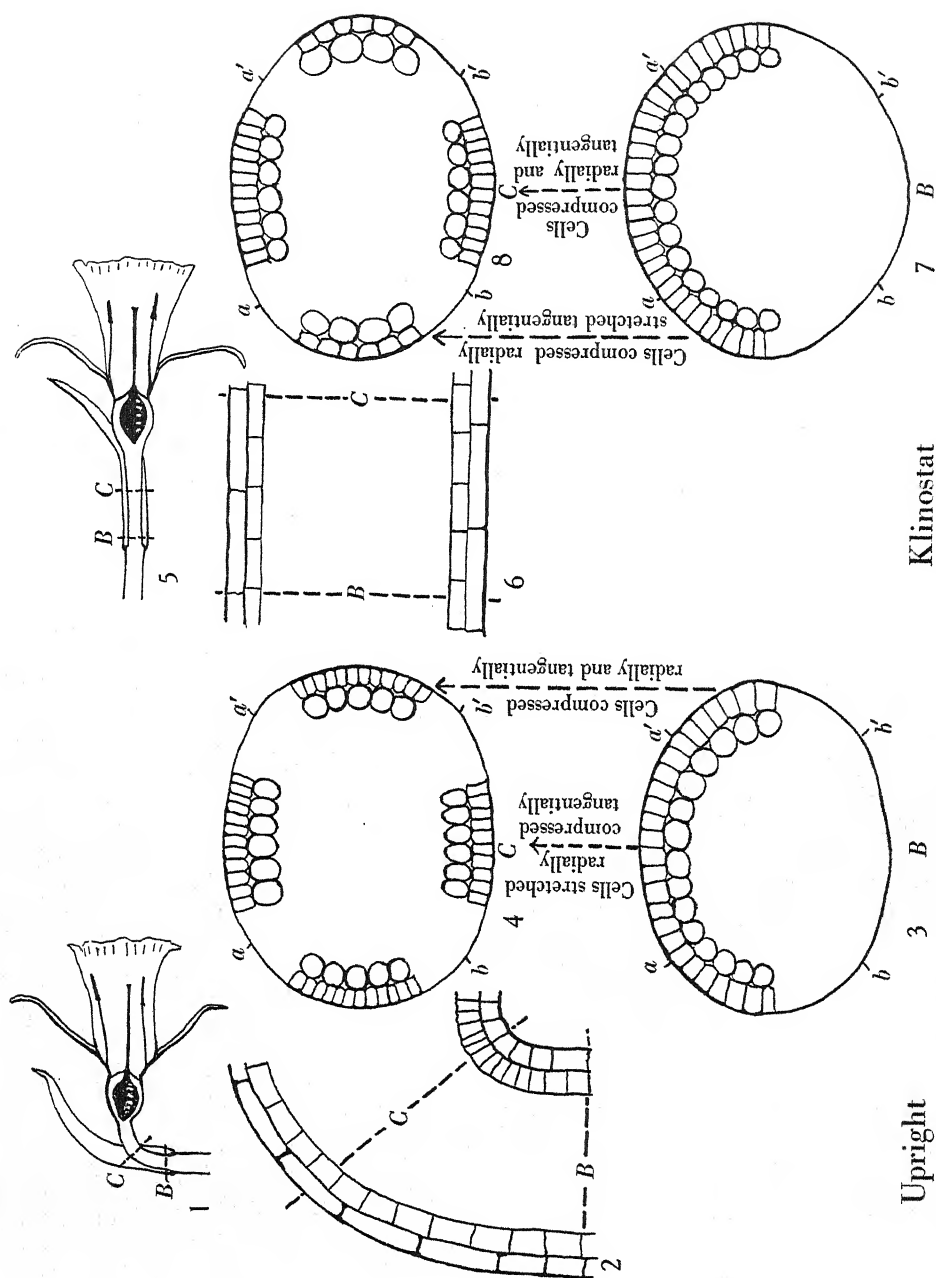


Fig. 2. *Narcissus pseudo-narcissus*. Diagram to show changes in shape of outer cells in the receptacle when the flower bends, and on a klinostat. 1. Normal flower. 2. Longitudinal section of receptacle of normal flower in the plane of the bend. 3. Transverse section of the receptacle of normal flower at B. 4. Transverse section of the receptacle of normal flower at C. 5. Flower grown on klinostat. 6. Longitudinal section of receptacle of klinostat flower in the plane of the shorter diameter. 7. Transverse section of klinostat flower at B. 8. Transverse section of klinostat flower at C.

Table I. *Narcissus pseudo-narcissus*. Flower

Average length of diameters of cells in microns.

Place of transverse section	Epidermis		Palisade		Statocytes	
	Upright	Klinostat	Upright	Klinostat	Upright	Klinostat
Mid peduncle:						
Radial diameter	25.0	16.25	23.75	30.75	45.63	35.83
Tangential diameter	15.0	15.0	20.0	22.5	28.66	28.75
Ratio	1.66	1.08	1.18	1.36	1.6	1.35
Receptacle at A, 5 mm. below sheath:						
Radial diameter	17.5	22.5	46.25	25.0	35.35	48.2
Tangential diameter	11.25	19.0	15.0	25.0	24.0	32.3
Ratio	1.55	1.18	3.04	1.0	1.42	1.49
Receptacle at B, base of sheath:						
Radial diameter	20.0	26.25	25.0	25.0	49.23	53.72
Tangential diameter	15.0	13.75	25.0	25.0	33.16	35.22
Ratio	1.33	1.9	1.0	1.0	1.37	1.52
Receptacle at C, mid-way between sheath and ovary:						
	(a-a') (b-b')		(a-a') (b-b')			
Radial diameter	23.0	23.25	28.25	23.75	42.93	41.92
Tangential diameter	11.25	11.5	21.5	22.75	31.08	29.06
Ratio	2.0	2.0	1.3	1.04	1.37	1.45
	(a-b) (a'-b')		(a-b) (a'-b')			
Radial diameter	15.75	16.75	20.0	22.5		
Tangential diameter	14.25	17.75	18.25	30.0		
Ratio	1.1	1.05	1.09	1.33		
Mid ovary:						
Radial diameter	25.0	20.0	17.0	17.3	39.2	34.56
Tangential diameter	15.0	15.0	36.75	28.7	27.75	22.63
Ratio	1.6	1.4	2.16	1.65	1.4	1.52
Base of perianth:						
Radial diameter	25.0	16.25	18.6	23.2	45.0	55.75
Tangential diameter	15.0	15.0	36.0	30.25	31.75	32.5
Ratio	1.66	1.08	1.93	1.3	1.41	1.71

a-a' and *a-b* denote different segments of receptacle (see Fig. 2 and explanation in text, p. 243).

Leaves of plants grown on the klinostat are of same average length and width as those grown upright. Measurements of the diameters of cells seen in transverse section show that in the klinostat leaves the cells of the epidermis, palisade tissue and statocytes are smaller than in upright leaves and the palisade cells are more nearly iso-diametric in shape. Seen in surface view the longitudinal diameters of epidermal cells are shorter in klinostat leaves, but the transverse diameters are the same. The average number of stomates, per square millimetre of the middle part of the leaf, is greater both on the inner and outer surfaces of klinostat leaves than upright ones. Three hundred and sixty counts were made for sixty leaves.

According to Salisbury (1927) a comparison of plants growing in dry and moist

air indicates that stomatal frequency is mainly dependent on the humidity of the environment and that total pore space per unit area of leaf is greater in dry than in moist conditions. On the other hand, he says that differences in stomatal frequencies may be almost entirely accounted for by the extent of growth of the epidermal cells and the spacing of the stomates rather than by differences in the numbers of stomata produced. To demonstrate this fact Salisbury uses the stomatal index, which may be calculated from the formula

$$I = \frac{S}{E+S} \times 100,$$

where S = number of stomates per unit area of leaf, E = number of epidermal cells for the same area, and I expresses the percentage proportion of epidermal cells which have been converted into stomata.

Plants growing in moist conditions would tend to have less stomata formed and lower indices than those growing under drier conditions.

If the stomatal index is calculated for *Narcissus* leaves and a comparison made between those grown on the klinostat and upright, the results show a higher value for klinostat leaves, 18.9 for the inner surface and 20.2 for the outer surface, as compared with 17.05 for the inner surface and 16.87 for the outer surface of upright leaves. This seems to indicate that the greater number of stomata in klinostat leaves is not entirely due to the difference in size of the epidermal cells, but may be also accounted for by a difference of humidity in the surrounding air. The constant movement of the plant on the klinostat would create a drier condition round the leaves, by constant movement of the air, than the upright leaves would have, growing normally. The drier condition might raise the stomatal index, in which case the alteration of the stomatal frequency is a secondary effect of growth on the klinostat. Table II shows the average measurements for *Narcissus* leaves.

Table II. *Narcissus pseudo-narcissus*. Leaves

Average diameters of cells in microns

Transverse section

	Epidermis		Palisade		Parenchyma	
	Upright	Klinostat	Upright	Klinostat	Upright	Klinostat
Radial diameter	25.0	22.5	50.0	44.25	70.0	50.0
Tangential diameter	16.5	14.0	21.5	24.0	43.5	32.5
Ratio	1.5	1.6	2.32	1.84	1.6	1.53

Epidermal cells in surface view

	Upright		Klinostat	
	Inner	Outer	Inner	Outer
Longitudinal diameter	192.5	224.0	180.0	185.0
Transverse diameter	21.75	23.0	22.0	23.0
Number of stomates per sq. mm.	47	43	62	58
Number of cells per sq. mm.	228	200	266	228
Stomatal index	17.05	16.87	18.9	20.2

Lupinus albus seedlings. In *Lupinus albus* seedlings the development of the statolith apparatus corresponds with the ontogeny of the latter in other seedlings as described by Hawker (1932, p. 126). In the very young seedling, when the root tip is emerging from the testa no starch is visible except in cells of the root tip. As the hypocotyl grows to 1.0 cm. in height, there are two layers of statocytes round the stele, consisting of the endodermis and adjacent cells of the cortex, and some statocytes in the pith, throughout the hypocotyl. Between 1.0 and 2.0 cm. in height, below the collet there are often starch grains in the endodermal cells; at the collet there are two or three layers of statocytes round the stele and scattered starch in the pith and cortex; at the middle of the hypocotyl there are two layers of statocytes round the stele, all pith cells containing falling starch and some of the cells of the cortex; at the cotyledonary node there is one layer of statocytes round the stele and falling starch in the pith cells and in some cells of the cortex. Between 2.0 and 3.5 cm. in height there are two layers of statocytes at the collet and no starch elsewhere; at the middle of the hypocotyl there are two layers of statocytes—the endodermis and the adjacent cells which contain small starch grains, and a little scattered starch in the pith and cortex. At the cotyledonary node there is one layer of statocytes round the stele and cotyledonary bundles. In hypocotyls 4.0 cm. in height and above that height, there is one layer of statocytes in the endodermis and sometimes starch in the cortex. It is impossible to make hard and fast divisions of length. As the cotyledons open and the epicotyl appears, the second layer of statocytes fades out as such. At first statoliths in the outer cells do not fall, but they decrease in size and disintegrate, then there is one row in the endodermis only, which disappears from the base upwards as the hypocotyl grows in length.

In seedlings grown on the klinostat there is no apparent difference in the size, quantity or distribution of statoliths in time or space.

The hypocotyls of seedlings grown on the klinostat are bigger in transverse section than those grown upright, but the shape of transverse sections is similar. Measurements of the stele in transverse section show a tendency to be slightly more elliptical in shape and larger in the plant grown on the klinostat than in the plant grown upright, in which case the shape of the stele is circular at the middle of the hypocotyl.

A comparison of the shape and size of the cells of the various tissues of the hypocotyls of seedlings of the same age grown on the klinostat with control seedlings shows, in transverse sections, an increase in the size of the cells of the cortex, endodermis and pith. The transverse sections compared were cut at the middle of the hypocotyl and the longitudinal sections in the upper half. In transverse section, the two outer layers of cells within the epidermis are stretched slightly tangentially and the cell walls are thicker than in the upright seedlings. Cells of the middle cortex and pith are larger in klinostat plants, but there is no change in their shape or in the thickness of the cell walls. Endodermal cells are slightly larger in area of the transverse section and more iso-diametric in shape on the klinostat, but do not show so much increase in size as the cortical cells. No difference has been found in the shape or size of the vascular tissue.

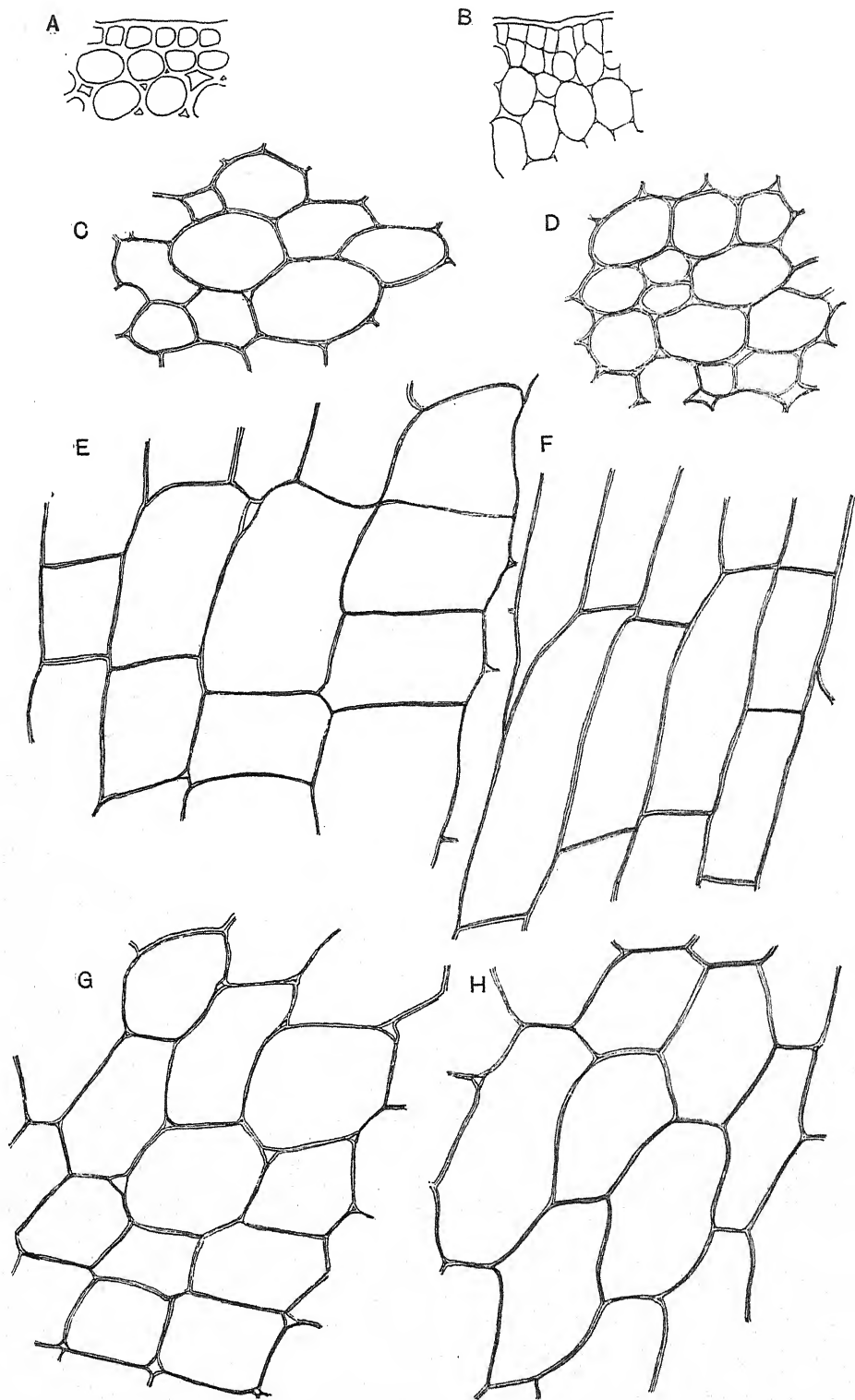


Fig. 3.

In longitudinal sections of hypocotyls cells of the cortex and pith are wider in the radial dimension in klinostat plants, but in tangential longitudinal sections there seems little difference in size. There is wide variation in the length of cells in one series and no definite difference one way between klinostat and control plants. Statocytes show very little difference in length or width in longitudinal section.

In comparing the radicles of klinostat and control seedlings the most evident difference seen in transverse sections is the alteration of shape in the outer cells of the cortex, which are stretched tangentially. Cells of the middle cortex are slightly smaller and rounder in shape on the klinostat, but in the pith there is no difference in the size or shape of the cells. In longitudinal sections cells of the cortex are definitely shorter, but there seems little difference in width in either radial or tangential direction.

Therefore the effect of the klinostat on hypocotyl and radicle appears to be different. In the hypocotyl, cells of the cortex are stretched tangentially at the outside and cells of the inner cortex and pith are bigger, but the same shape as in upright plants. In the radicle, the cortical cells are shortened in the longitudinal direction, and seen in transverse section they are smaller on the klinostat than in upright plants.

Results for *Lupinus albus* are recorded in Table III. The shape of cells is compared by ratios of the long and short diameters of the cells.

Asplenium bulbiferum fronds. The statolith apparatus of *Asplenium bulbiferum* fronds has been described by Dr Prankerd (1922). She describes the chlorostatenchyma as dying away in the lower part of the petiole as the xylem becomes lignified and sclerenchyma is developed, and having disappeared altogether before the end of the adolescent phase. In the fronds examined by the writer, statenchyma has been found reaching to the base of the petiole and in some cases it had not disappeared when the frond was almost unfolded. The sclerenchyma in the late infant stage of the frond, before any pinnae are unfolded below the apical coil, is found at the base of the petiole forming two bands round the cortex beneath the epidermis, separated by a few parenchymatous cells. At this stage the cells of the cortex outside the endodermis of the meristeles become lignified first in the transverse and later the inner tangential walls. When the frond is unfurled the bands of sclerenchyma, six cells wide, round the petiole extend from the base to the base of the pinnae; above, in the lamina, a short band of sclerenchyma persists, usually abaxially, almost to the tip of the frond. The cortical cells round the endodermis of the meristeles are lignified, sometimes two cells deep, from the base of the petiole to the upper half of the lamina.

Legend to Fig. 3.

Fig. 3. *Lupinus albus*. Cells from seedlings of the same age grown upright and on the klinostat. $\times 167$. A. Epidermis of klinostat hypocotyl in transverse section. B. Epidermis of upright hypocotyl in transverse section. C. Cortex of klinostat hypocotyl in transverse section. D. Cortex of upright hypocotyl in transverse section. E. Cortex of klinostat hypocotyl in longitudinal radial section. F. Cortex of upright hypocotyl in longitudinal radial section. G. Cortex of klinostat radicle in longitudinal radial section. H. Cortex of upright radicle in longitudinal radial section.

Table III. *Lupinus albus*. *Radicle*

Transverse section. Average length of diameters of cells in microns

	Outer cortex		Middle cortex		Pith	
	Upright	Klinostat	Upright	Klinostat	Upright	Klinostat
Radial diameter	45.3	25.3	66.9	56.9	67.7	70.5
Tangential diameter	27.7	30.5	47.8	46.3	47.8	47.8
Ratio	1.63	1.2	1.39	1.22	1.44	1.47

Radial longitudinal section of cortex

	Upright	Klinostat
Radial diameter	70.2	75.6
Long diameter	142.0	128.6

Tangential longitudinal section of cortex

	Upright	Klinostat
Tangential diameter	60.8	67.4
Long diameter	147.6	136.0

Lupinus albus. *Hypocotyl*

Transverse section

	Outer cortex		Middle cortex		Endodermis		Pith	
	Upright	Klinostat	Upright	Klinostat	Upright	Klinostat	Upright	Klinostat
Radial diameter	29.8	29.0	67.6	98.3	27.1	33.6	81.9	100.8
Tangential diameter	28.7	42.0	46.7	60.9	21.3	28.1	59.9	70.1
Ratio	1.03	1.44	1.44	1.61	1.27	1.19	1.36	1.43

Radial longitudinal section of cortex

	Upright	Klinostat
Radial diameter	73.9	88.5
Long diameter	226.8	241.7

Tangential longitudinal section of cortex

	Upright	Klinostat
Tangential diameter	54.6	65.2
Long diameter	225.0	164.5

In fronds grown on the klinostat for periods of three to four weeks no difference has been found in the development or arrangement of the statenchyma, but there is a marked alteration in the amount of sclerenchyma formed, and in the size of the cortical cells. In the young frond, as it unfurls, the band of sclerenchyma at the base of the petiole is narrower, the cell walls are thinner and the lumen of the cells larger in fronds grown on the klinostat, and in the cortical cells round the meristeles the inner walls and corners of the cells are lignified at the base of the petiole only, instead of halfway up the petiole, as in upright fronds. In mature fronds grown on

the klinostat the external bands of sclerenchyma are narrower, and extend for the basal 2-3 cm. of petiole only; above this a short band is found, but this does not extend at all above the base of the lamina (see Fig. 4). The walls of the sclerenchymatous cells are thinner and the lumen bigger in transverse section. In longitudinal

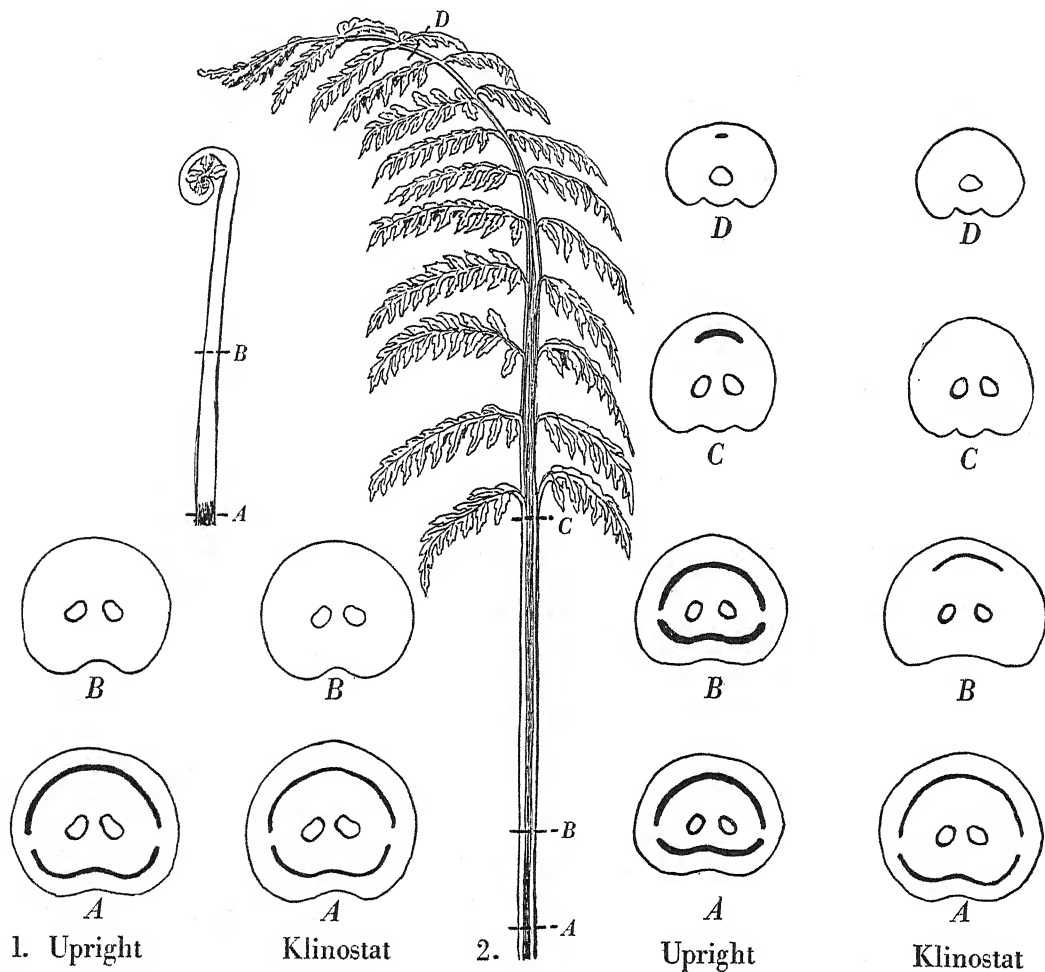


Fig. 4. *Asplenium bulbiferum*. 1. Late infant stage of frond. Transverse sections of petiole in planes A and B for upright and klinostat fronds. 2. Mature stage of frond. Transverse sections of petiole in planes A-D respectively for upright and klinostat fronds. Sclerenchyma is marked in black.

section the cells appear half as long and more than twice as wide in plants grown on the klinostat than upright ones. The cells round the meristeles are thickened as in upright plants from the base of the petiole up to the upper part of the lamina.

On the klinostat, cells of the cortex are larger in transverse section. In longitudinal section the cells of the cortex are much wider and shorter in klinostat fronds than in upright ones, averaging $107.5 \times 100\mu$ as compared with $137.5 \times 52.5\mu$ at the

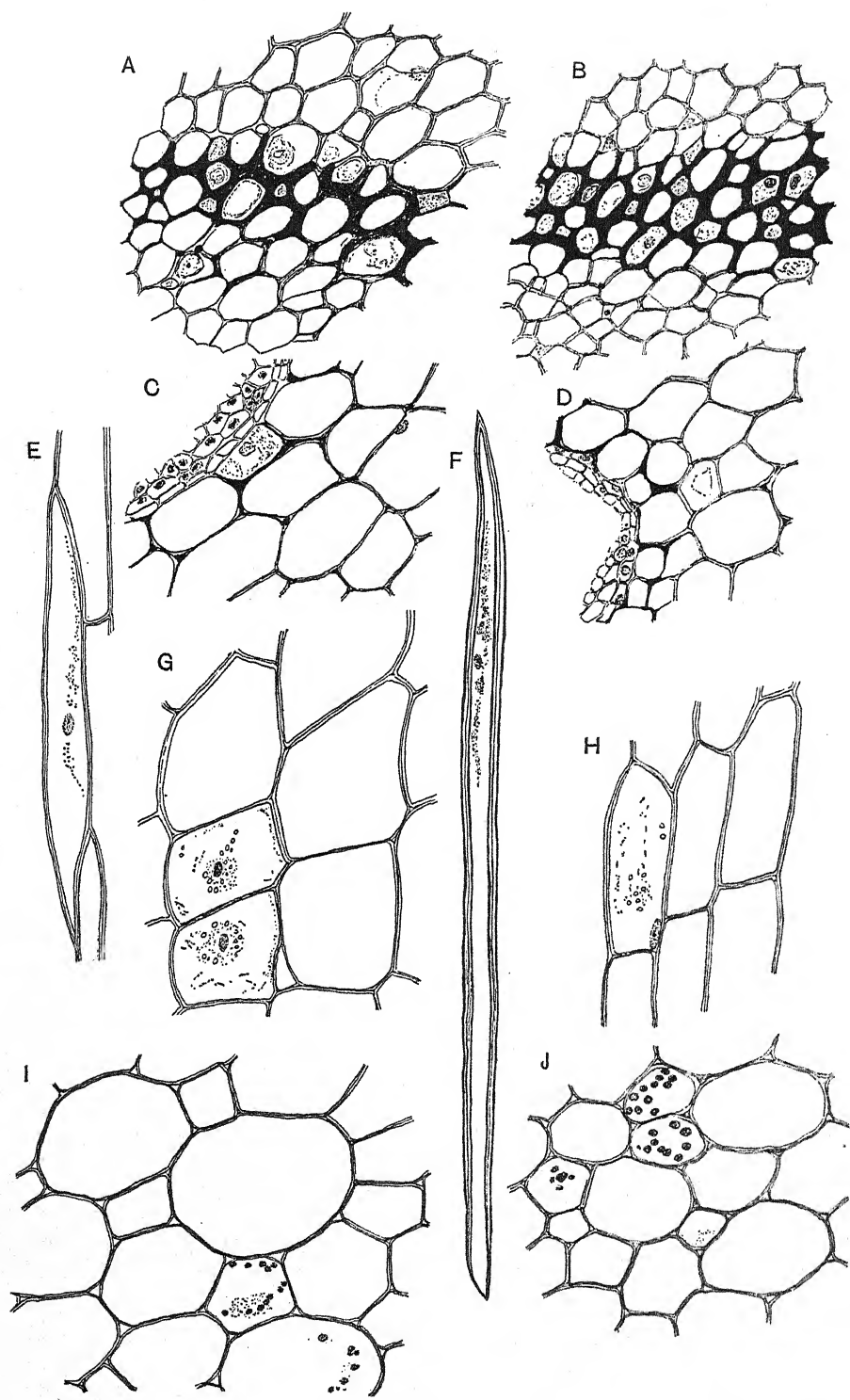


Fig. 5.

base of petioles of mature fronds. The stretching of the cells in this way might account for the more rapid unrolling of the apical coil noted in fronds grown on the klinostat (Brain, 1935). The epidermal cells are stretched tangentially.

Figs. 4 and 5 illustrate the differences described above. Measurements are recorded in Table IV.

Table IV. *Asplenium bulbiferum*. Fronds

Average length of diameters of cells in microns

	Transverse section			
	Epidermis		Cortex statenchyma	
	Upright	Klinostat	Upright	Klinostat
Late infant stage. Base of frond				
Radial diameter	19.5	16.25	49.25	73.25
Tangential diameter	17.0	24.0	77.5	90.5
Ratio	1.14	1.47	1.57	1.23
Mature stage. Base of frond				
Radial diameter	22.25	19.25	62.5	101.25
Tangential diameter	21.5	24.75	88.25	118.75
Ratio	1.03	1.28	1.41	1.17

Radial longitudinal section of cortex		
	Upright	Klinostat
Radial diameter	52.5	100.0
Long diameter	137.5	107.5

DISCUSSION

Czaga (1935) showed that applications of growth hormone paste in a ring round the hypocotyl of seedlings caused stretching of the cells transversely and increased thickness of the organ according to the concentration of the growth hormone in the paste. On p. 202 he gives a striking illustration of the longitudinal section of *Helianthus* hypocotyl treated with a ring of growth hormone paste. The effect of the klinostat in producing increase in size in cells of the stem indicates a comparable effect, either as a result of re-distribution of growth substance round the stem or of increased production from the continuous all-sided gravitational stimulus. Dijkman (1934) has found that in *Lupinus albus* growth substance is found throughout the

Legend to Fig. 5.

Fig. 5. *Asplenium bulbiferum*. Sections of base of petiole in mature stage of fronds grown on the klinostat and upright. $\times 167$. A. Transverse section of outer cortex showing sclerenchyma in klinostat petiole. B. Transverse section of outer cortex showing sclerenchyma in upright petiole. C. Transverse section showing sclerenchyma round meristele in klinostat petiole. D. Transverse section showing sclerenchyma round meristele in upright petiole. E. Sclerenchyma element from outer cortex of klinostat petiole. F. Sclerenchyma element from outer cortex of upright petiole. G. Cells of cortex of klinostat petiole in longitudinal radial section. H. Cells of cortex of upright petiole in longitudinal radial section. I. Cells of cortex of klinostat petiole in transverse section. J. Cells of cortex of upright petiole in transverse section.

growing region of the hypocotyl and is probably formed throughout, and on geotropic stimulation redistribution occurs resulting in a higher percentage of auxin on the lower side of a horizontal hypocotyl. Thus it appears that on the klinostat it is a redistribution and not increased production of growth substance which causes the alteration in the size and shape of cells. Continuous revolution in a horizontal position would keep the auxin supplied to the tangential walls and result in an increase in size all round the stem which, when one-sided, would result in a curvature. This is the effect shown in *Lupin* hypocotyls and in petioles of *Asplenium bulbiferum*.

In radicles of *Lupinus albus*, cells of the cortex are slightly smaller in transverse section and in longitudinal section the longitudinal walls are shorter on the klinostat than in upright plants. An opposite effect of the redistribution of auxin is therefore shown in root and shoot.

In *Narcissus* flowers the bend of the receptacle does not occur on the klinostat, but changes in the shape of cells of the external tissues take place.

Zollikofer (1935) attributes the difference in length of the dorsal and ventral halves of curved flower stalks to unequal elastic pressure on the two halves dependent on unequal growth hormone distribution. On the klinostat the normally unequal distribution would be equalized and alteration in the distribution of elastic pressure might result, which possibly explains the tangential stretching of the cells on the klinostat, which are compressed as bending occurs normally. It is certainly significant that these changes coincide with a maximum development of statoliths in the receptacle, and this points to the possibility of some link between the statolith apparatus and those changes of elasticity of the cell walls which have been ascribed to the action of growth hormone.

In *Narcissus* leaves the cells are shorter in longitudinal section and smaller in transverse section on the klinostat. In petioles of *Asplenium bulbiferum* the cortical cells seen in longitudinal section are shorter, but also much wider than in upright plants. The marked difference in the development of sclerenchyma in the petiole on the klinostat raises a point of interest. Bower (1923), dealing with the distribution of mechanical tissues in plants, regards them as adaptive and acquired in course of descent according to the requirements of the plant. He says the structure is, as a rule, hereditary, although the conditions to which developing parts are exposed may have an influence on determining the quantity of sclerotic tissue formed in the individual part. The effect of the klinostat in reducing the amount of sclerenchyma formed and producing thinner walled elements indicates a new piece of evidence for the influence of growth hormones on cell wall plasticity. Bücher (1906) described greater thickening of walls and smaller lumen of cells of collenchyma, bast, and wood on the upper side and larger lumen and less thickening than normally on the lower side when stems were kept in a horizontal position and prevented from bending geotropically. Heyn (1934) has shown that geotropic stimulus causes the under side of horizontal stems to be more plastic and the upper side less plastic than normal, the change in plasticity being influenced by the growth hormone distribution. One may suppose, in the normal petiole, that sclerenchyma is formed as the cells become

older and lose their plasticity. On the klinostat the growth hormone supply is kept up by continual geotropic stimulation and the ageing and thickening process is retarded. The cells also remain plastic, are stretched transversely, and are much shorter than normal fibres.

The development of dicotyledonous embryos in *Ceratozamia*, when rotated on a klinostat, described by Dorety, and the change in structure of *Marchantia* sporophytes ("Brutkörper") described by Czapek (1898) may now be explainable in the light of the growth hormone theory, for it seems possible that constant rotation on a klinostat may cause alterations in the plasticity and development of cells as a result of a constant distribution of growth hormone by prolonged all-sided geotropic stimulation.

SUMMARY

1. The anatomy of flowers and leaves of *Narcissus pseudo-narcissus*, fronds of *Asplenium bulbiferum* and seedlings of *Lupinus albus* has been compared for normal plants and plants grown on a horizontal klinostat.

2. In flowers of *Narcissus pseudo-narcissus*, growth on a klinostat does not affect the development or distribution of statocytes, but changes occur in the shape and size of cells of the outer tissues of the peduncle and receptacle.

3. In leaves of *Narcissus* grown on the klinostat, cells of epidermis and palisade tissue are smaller in transverse section and epidermal cells are shorter longitudinally than in upright plants. The average number of stomates per square millimetre is greater on both outer and inner surfaces of klinostat leaves than in upright ones.

4. In *Lupinus albus* seedlings no difference has been found in the size, quantity, or distribution of statoliths, in time or space when seedlings are grown on the klinostat. Transverse sections of hypocotyls of klinostat seedlings show that cells of cortex, endodermis, and pith are larger. In longitudinal section cells of the cortex and pith are wider radially, and longer than in upright plants. In longitudinal section cells of the cortex and pith of radicles are shorter in klinostat than upright seedlings. Thus the growth on the klinostat has an opposite effect on the cells of hypocotyl and radicle.

5. In petioles of *Asplenium bulbiferum* no difference has been found in the amount or distribution of statenchyma. In klinostat fronds much less sclerenchyma is formed and the sclerenchymatous cells are shorter, and have larger lumina and less thickened walls than in upright fronds, also cells of the cortex are much wider and shorter in longitudinal section and bigger in transverse section than in upright fronds.

My thanks are due to Dr T. L. Prankerd, F.L.S., for her interest and kindness in reading this manuscript.

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STUDIES ON THE SULPHUR METABOLISM OF PLANTS

II. THE EFFECT OF NITROGEN SUPPLY ON THE AMOUNTS OF PROTEIN SULPHUR, SULPHATE SULPHUR AND ON THE VALUE OF THE RATIO OF PROTEIN NITROGEN TO PROTEIN SULPHUR IN LEAVES AT DIFFERENT STAGES DURING THE LIFE CYCLE OF THE PLANT

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(With 3 figures in the text)

INTRODUCTION

IN a previous paper (Wood & Barrien, 1939) an experiment was described wherein the amounts of sulphur fractions were determined in plants subjected to increasing doses of ammonium salts. In this experiment the ratio of protein nitrogen to protein sulphur showed a tendency to increase with increased ammonium treatment; but the amount of variation in both quantities was so small that, with the method of analysis employed, it could not be said with certainty that such change in the ratio was significant.

Therefore we decided to determine the value of this ratio at stages during the life cycle of a plant and to employ a more accurate method for determining protein sulphur. We are greatly indebted to Dr A. H. K. Petrie of the Waite Research Institute who placed at our disposal dried material of leaves of Sudan grass (*Andropogon sudanensis*, Leppan and Bosman) which had been used in an experiment concerned with the relation of ontogeny to nutrition (Ballard & Petrie, 1936; Petrie, 1937; Williams, 1936, 1938).

EXPERIMENTAL

The leaf material was obtained from plants subjected to three different nitrogen treatments; seven harvests from each treatment were taken during the life cycle of the plant. Details of the experimental procedure have been described by Ballard & Petrie (1936).

In brief, the three treatments were: treatment I, 0.75 g. NaNO_3 per pot; treatment II, 2.25 g. NaNO_3 per pot; treatment III, 4.50 g. per pot. Other nutrients, including sulphate, were supplied at constant initial concentration. The first harvest

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took place 9 days after planting and before the nutrients were applied; subsequent harvests took place 21, 33, 40, 55, 76, and 103 days after planting respectively.

The analytical procedure was as follows: A weighed amount, not exceeding 2 g. of the carefully dried and finely ground leaf material was extracted with 100 ml. distilled water at 60° C. for 1 hr., after which it was boiled for 5 min. The mixture was cooled to 30° C. and 40 ml. of a 4 % tannin solution in 0.5 % HCl added. The mixture was allowed to stand for 15 min. and then filtered and the residue washed with 1 % tannin solution. Sulphate sulphur was determined in the filtrate by precipitation with BaCl₂ after acidifying with HCl.

The residue was dried and, including the filter paper, pressed into a compact pellet which was then ignited in oxygen at 200 lb. pressure in an Emerson calorimeter bomb. After cooling, the bomb was thoroughly washed out and the resulting solution evaporated, after addition of bromine water, to a volume of about 100 ml. After filtration, appropriate dilution and acidification, the sulphate formed during the oxidation was precipitated with BaCl₂. All weighings were carried out on materials and vessels allowed to equilibrate with air in the balance case. Duplicate analyses of the leaf material all agreed within 1 %.

RESULTS

The results of analysis have been expressed as absolute values (grams of substance per plant) and as relative values (percentage of dry matter). These are given in Tables I and II and are expressed graphically in Figs. 1 and 2. In addition, relevant figures for protein nitrogen and amount of dry matter from Petrie's data (1937) are given in Tables I and II.

Table I. *Absolute amounts* of dry-matter protein sulphur, protein nitrogen, sulphate sulphur and water in leaves*

Treatment I					
	Mean dry wt. per plant	Prot. N	Prot. S	SO ₄ S	Water
Harvest 1	0.0023	0.0667	0.00368	0.00104	0.0114
2	0.0251	0.964	0.0622	0.0113	0.129
3	0.288	7.95	0.555	0.121	1.40
4	0.773	17.01	1.22	0.342	3.18
5	1.63	16.46	1.23	†	4.19
6	1.65	10.96	†	†	2.65
7	1.39	4.64	†	†	0.89

Treatment II					
	Mean dry wt. per plant	Prot. N	Prot. S	SO ₄ S	Water
Harvest 1	0.0023	0.0667	0.00368	0.00104	0.0114
2	0.0234	0.934	0.0577	0.0108	0.119
3	0.293	9.35	0.603	0.123	1.46
4	1.000	29.80	1.94	0.430	4.41
5	3.05	50.33	3.66	0.702	8.01
6	2.56	31.23	2.77	0.461	5.24
7	2.72	17.84	1.88	0.625	2.36

Table I (continued)

Treatment III					
	Mean dry wt. per plant	Prot. N	Prot. S	SO ₄ S	Water
Harvest 1	0.0023	0.0667	0.00368	0.00104	0.0114
2	0.0199	0.770	0.0434	0.00835	0.099
3	0.258	8.46	0.529	0.0748	1.26
4	0.928	28.49	1.81	0.260	4.15
5	2.82	66.83	4.45	0.535	8.43
6	3.61	61.74	4.95	0.772	9.47
7	3.30	37.62	3.04	0.726	4.92

* Amounts of water in grams, of other fractions in milligrams, per plant.
 † Insufficient material for analysis.

Table II. Percentage amounts of protein sulphur, protein nitrogen, sulphate sulphur and water* in leaves

Treatment I					
	Prot. N	Prot. S	SO ₄ S	Water	Ratio Prot. N Prot. S
Harvest 1	2.90	0.160	0.045	491	18.1
2	3.84	0.248	0.045	512	15.5
3	2.76	0.193	0.042	490	14.3
4	2.20	0.158	0.043	406	13.9
5	1.10	0.075	†	253	13.5
6	0.664	†	†	160	—
7	0.334	†	†	62	—

Treatment II					
	Prot. N	Prot. S	SO ₄ S	Water	Ratio Prot. N Prot. S
Harvest 1	2.90	0.160	0.045	491	18.1
2	3.99	0.247	0.046	509	16.2
3	3.19	0.206	0.042	498	15.5
4	2.98	0.194	0.043	441	15.3
5	1.65	0.120	0.023	268	13.7
6	1.22	0.108	0.018	205	11.3
7	0.656	0.069	0.023	92	9.5

Treatment III					
	Prot. N	Prot. S	SO ₄ S	Water	Ratio Prot. N Prot. S
Harvest 1	2.90	0.160	0.045	491	18.1
2	3.87	0.218	0.042	496	17.7
3	3.28	0.205	0.029	490	16.0
4	3.07	0.195	0.028	444	15.7
5	2.37	0.158	0.019	299	15.0
6	1.71	0.137	0.022	273	12.5
7	1.14	0.092	0.022	149	12.4

* The amounts for water are the amounts associated with 100 g. dry weight.
 † Insufficient material for analysis.

Absolute drifts

Protein sulphur (Fig. 1). Protein sulphur in each treatment rises to a maximum value and thereafter declines. The most striking feature of the curves for amount of protein sulphur plotted against time is that they follow the trend of the curve for amount of dry matter.

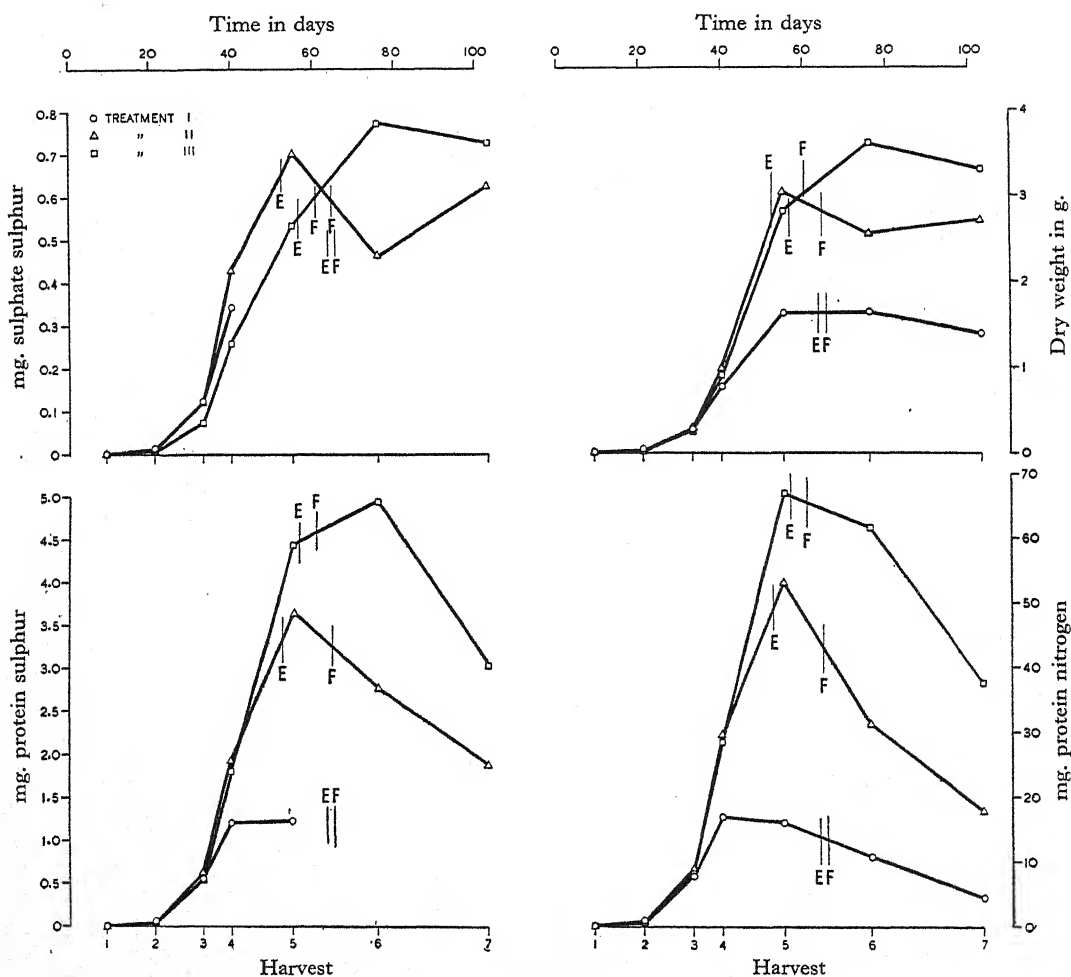


Fig. 1. Absolute amounts of sulphate sulphur, dry matter, protein sulphur, and protein nitrogen of total leaves per plant. From data of Table I. *E* indicates times of exsertion of inflorescences and *F* time of flowering.

Although the form of the curve for amount of protein sulphur resembles in a general way that for protein nitrogen, yet the positions of the maxima are not coincident except in the case of treatment II. In treatments I and III the maximum for protein sulphur is later than for protein nitrogen.

Inorganic sulphate sulphur (Fig. 1). The curves for amount of sulphate sulphur plotted against time follow in each case the trends of the curves for amounts of dry matter, water and protein sulphur.

Relative drifts

Protein sulphur (Fig. 2). The curves for amount of protein sulphur resemble those for protein nitrogen; the amount of protein sulphur reaches a maximum at

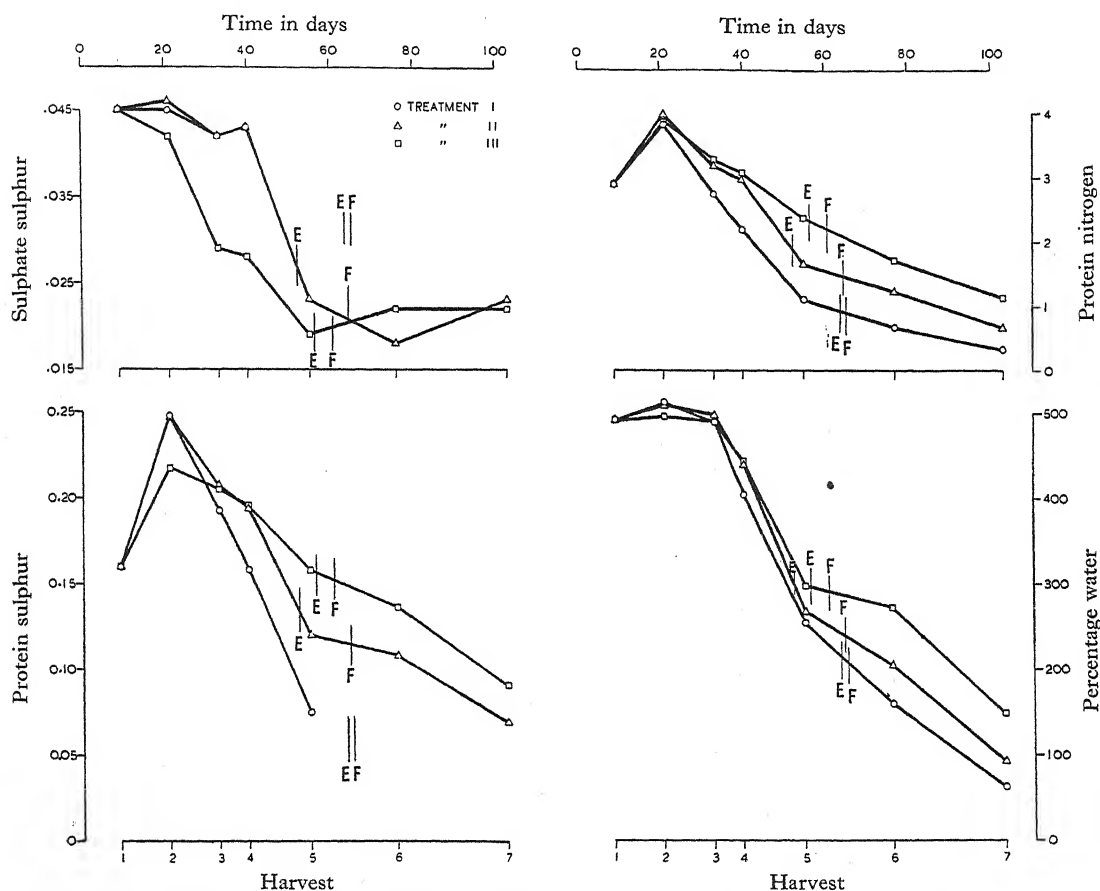


Fig. 2. Amounts of sulphate sulphur, protein nitrogen, protein sulphur, and water in grams per 100 grams dry matter, from data of Table II. *E* indicates times of exsertion of inflorescences and *F* time of flowering.

harvest 2 and thereafter declines. The percentage decrease in protein sulphur with time is less than in the case of protein nitrogen. The close connexion between synthesis of protein sulphur and nitrogen supply is also seen at harvest 2 of the highest nitrogen treatment: protein sulphur, like protein nitrogen and also dry matter, is lower in amount than in the other treatments owing to a temporary reduction in growth.

Inorganic sulphate sulphur (Fig. 2). In treatments I and II the percentage of sulphate sulphur shows little change in harvests 1-4, thereafter it decreases. In treatment III there is a slow fall in value between harvests 1-2 and thereafter a more rapid fall.

Protein nitrogen/protein sulphur ratios. These are illustrated in Fig. 3. The values for this ratio show two definite trends. First, the value of the ratio increases with treatment and second, the value of the ratio decreases with time in each treatment.

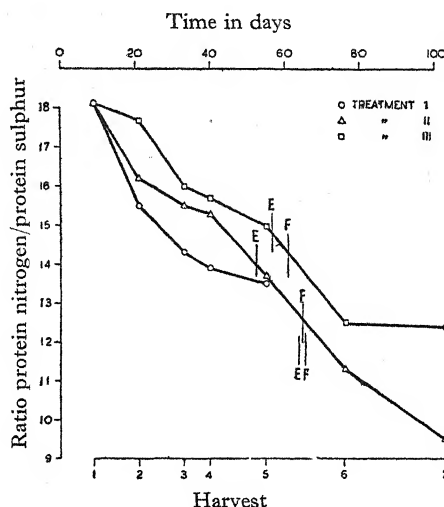


Fig. 3. Values of the ratio of protein nitrogen to protein sulphur plotted against time. From data of Table II.

DISCUSSION

The relation of protein sulphur to protein nitrogen and to the amounts of dry matter and water

The increase in amount of protein sulphur at each harvest with increased nitrogen treatment (Figs. 1, 2) when the initial amount of sulphate supplied remains unchanged confirms the results already described by Wood & Barrien (1939).

In explanation of the increase in value of the protein nitrogen/protein sulphur ratio with treatment, which is evident at each harvest, we are confronted with two alternatives. Either there is only one protein which varies in constitution or there is more than one protein, each with a different sulphur content, and the relative proportions of these proteins change with treatment.

The latter alternative appears the more likely, for Foreman (1938) and Bawden & Pirie (1938) have demonstrated the existence of several proteins with distinct physical properties in leaves of normal plants. The explanation we advance is that with increased nitrogen treatment one or more proteins, relatively rich in sulphur, increase in amount, but at the same time are diluted with one or more other proteins containing relatively less sulphur.

For the present this explanation applies only to the special case of Sudan grass. It is possible that in some species the ratio of protein nitrogen to protein sulphur may decrease in value with treatment owing to dilution of a protein with another containing relatively more sulphur. It is also possible, as in the case of vicilin and legumelin, reserve proteins of the pea seed, that the dilutant may contain only a very small amount of sulphur in the molecule. A further possibility is that both diluting and diluted proteins may have the same sulphur content.

From this viewpoint the decrease in value with time of the protein nitrogen/protein sulphur ratio in Sudan grass could be ascribed to the more rapid decrease in the sulphur-poor protein compared with the sulphur-rich protein. It is tempting in the present case to consider the sulphur-rich protein as the more stable cytoplasmic protein and the sulphur-poor protein as a reserve protein.

This idea receives some support from the data for the absolute amounts of protein sulphur and protein nitrogen and their relation to the amount of dry matter. The trends of the curves for protein sulphur follow those for amounts of dry matter and the positions of the maxima are coincident, whereas the maximum value for protein nitrogen is attained earlier than that of protein sulphur. If protein sulphur is regarded as a measure of the cytoplasmic protein, then increase in dry matter might be expected to be proportional to increase in protein sulphur. It is obvious, however, that all that can be said with certainty is that amount of protein sulphur is more highly correlated with amount of dry matter than is protein nitrogen.

Of the factors contributing to decrease in amount of protein sulphur the more important appear to be formation of the inflorescences (*vide* Fig. 1), decrease in water content (Petrie & Wood, 1938) and, possibly, depletion of the external supply of nitrogen: it is not probable that the supply of sulphate was depleted to such an extent that it became limiting, since at harvest 7 the amount of sulphate on a dry-weight basis had fallen to only one-half of the amount originally present.

Inorganic sulphate sulphur

Rate of growth of the leaves is probably the greatest single factor determining the amount of sulphate sulphur present in the leaves. The absolute amount of sulphate sulphur is greater the greater the dry weight of the leaves. In absence of data for stems and roots no certainty can be reached, but the decline in percentage content of sulphate sulphur is probably due to a partial depletion of the external supply which would bring about a decline in the rate of import of sulphate and consequently a decline in the percentage sulphate content.

SUMMARY

This paper describes the changes in amounts of protein sulphur and of sulphate sulphur during the life cycle of grass plants (*Andropogon sudanensis* Leppan & Bosman) which received three different initial supplies of nitrogen, the initial supply of sulphate remaining the same.

Increased nitrogen supply caused an increase in the amount of protein sulphur. As in the case of protein nitrogen, the highest nitrogen treatment caused at first a

depression in amount of protein sulphur due to an effect of treatment on growth rate.

The absolute content of protein sulphur rises to a maximum and thereafter decreases. The position of this maximum is coincident with the maxima for amounts of dry matter and of water, but is not coincident with the maximum for protein nitrogen which is attained earlier. On a relative basis, after an initial rise, the amount of protein sulphur decreases throughout the life cycle, although the rate of decrease is less rapid than that of protein nitrogen.

The latter effect is seen in the value of the ratio of protein nitrogen to protein sulphur. This ratio decreases in amount throughout the life cycle of the plant, and at any harvest is higher the higher the nitrogen treatment.

It is suggested that with increased nitrogen treatment, protein sulphur increases in amount but at the same time is diluted with a protein containing relatively less sulphur; and also that the latter protein is utilized more rapidly within the plant than is the sulphur-rich protein. Various factors contributing to the decrease in amount of protein sulphur are discussed.

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STUDIES ON THE SULPHUR METABOLISM OF PLANTS

III. ON CHANGES IN AMOUNTS OF PROTEIN SULPHUR AND SULPHATE SULPHUR DURING STARVATION

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(With 3 figures in the text)

INTRODUCTION

IN paper I of this series (Wood & Barrien, 1939) it was shown that if cystine is applied to the soil in which grass plants are growing, then there is no change in the amounts of protein sulphur and of cystine in the leaves, but the amount of sulphate sulphur increases. It was considered probable that inorganic sulphate was the final product of protein katabolism in the plant.

Mothes & Specht (1934) consider that in the katabolism of protein, sulphur-containing intermediary compounds are oxidized not to inorganic sulphate but to ethereal sulphates, similar to the phenolsulphonic acids produced in sulphur metabolism in the animal body. They look upon these conjugated sulphates as forming a "sulphur reserve" in the plant.

In order to study protein katabolism further, we describe in this paper the results of two experiments with grass plants during starvation attained by placing the plants in a dark room under constant conditions over a period of fifteen days.

EXPERIMENTAL

Material

Exp. 1. Seeds of a pure line of *Lolium multiflorum* Lam. were planted on 26 June 1935 and the seedlings transferred to pots containing 3.50 kg. of washed sand on 8 July. The nutrient solutions applied were the same as those described by one of us (Petrie & Wood, 1938 *a*) in preparation of plants for investigations on nitrogen metabolism. Nutrients were applied on 22 July, 8 August, 3 September and 4 October. Throughout the growing period the water content of the sand was maintained at 70 % saturation. Sixteen pots were placed in a constant temperature (16° C.) dark room with adequate ventilation on 28 October and samples of these pots were withdrawn and harvested on the 1st, 4th, 9th and 15th day after placing in darkness. Throughout the experimental period the water content of the sand was maintained at 70 % saturation.

Exp. 2. Sorted seeds of a pure line of Wimmera rye grass (*Lolium subulatum* Vis.) were sown on 6 May 1937 and the seedlings transferred to pots containing 3.50 kg.

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of washed sand on 7 June. The nutrient solutions used were the same as in Exp. 1. On account of the large size of the seedlings the first culture solution was added to the sand before transplantation. The second culture solution was added on 26 June and the last on 14 July. Throughout the growing period the sand in the pots was maintained at 70 % saturation with water. On 17 September eighteen pots con-

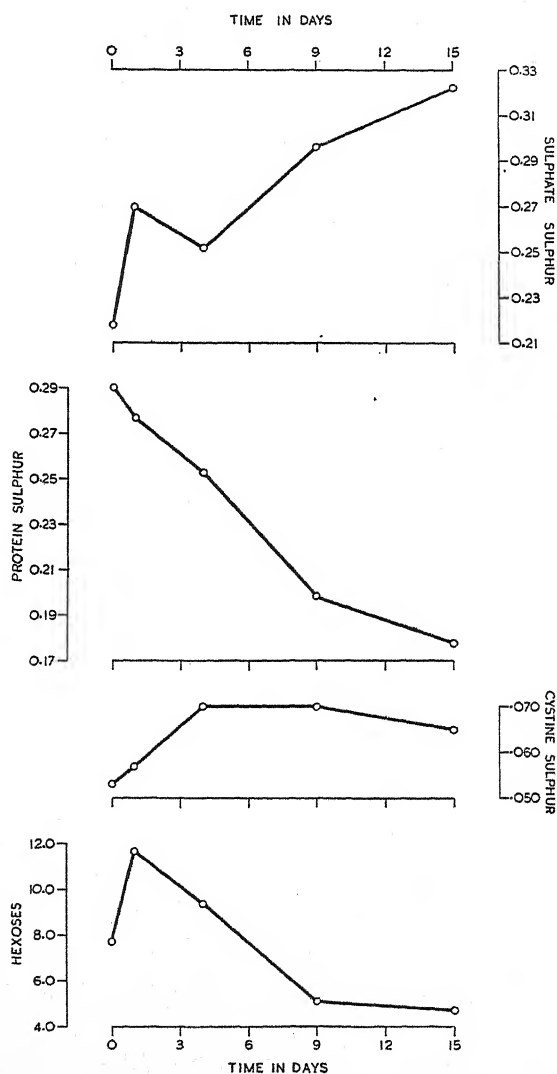


Fig. 1. Amounts of protein sulphur, inorganic sulphate sulphur, cystine sulphur and hexoses in grams per 100 g. dry matter. From data of Table I, Exp. 1.

taining even plants were placed in a constant temperature (16° C.) dark room and samples were withdrawn and harvested on the 3rd, 5th, 8th, 12th and 15th day after placing in darkness. The water content of the sand was maintained at 70 % saturation throughout the experiment.

Analytical procedure

The harvest as well as the analytical procedure differed in the two experiments.

In Exp. 1 leaves only were harvested and aliquots of the chaffed fresh leaves were immediately ground with sand and tannin solution, and fractions analysed according to the technique described by Wood & Barrien (1939). Protein sulphur

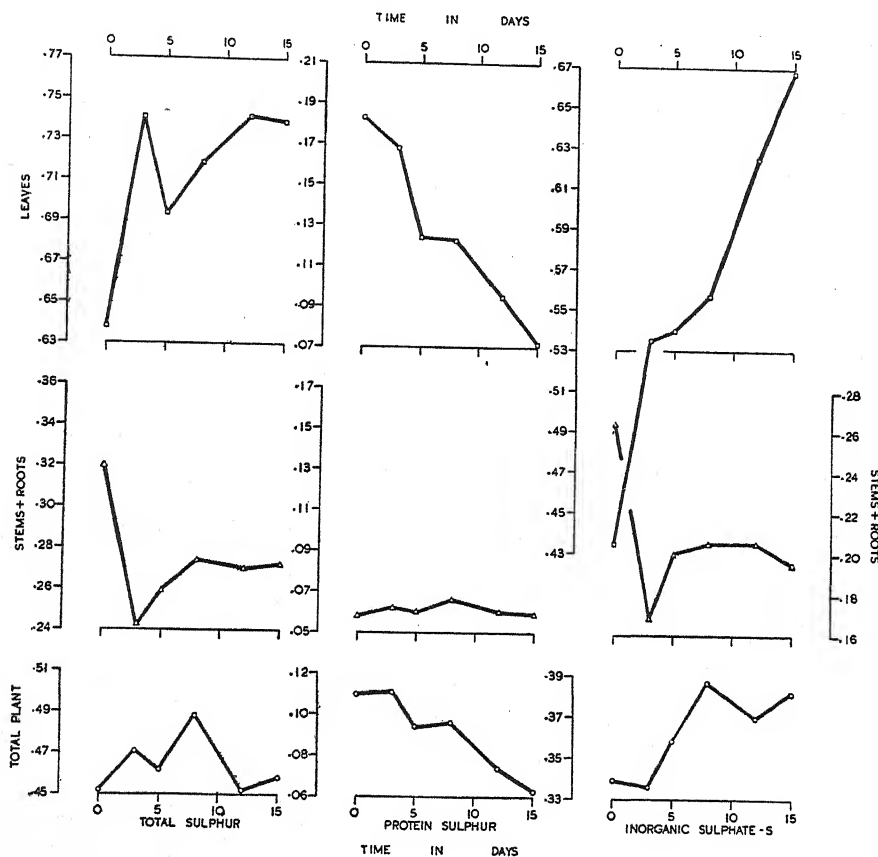


Fig. 2. Amounts of total sulphur, protein sulphur, and inorganic sulphate sulphur in grams per 100 g. dry matter in leaves, stems *plus* roots and total plant. From data of Table II, Exp. 2.

was determined by the method of Benedict-Denis. Additional aliquots of the fresh material were ground with $2/3 N$ tungstic acid and in the filtrate cystine was determined by Lugg's (1932) modification of the Folin-Marenzi method and hexoses by the method of Benedict-Wu.

In Exp. 1 it was considered possible that soluble sulphur-containing compounds in the leaves might have changed in amount owing to translocation during the course of the experiment. In Exp. 2, therefore, leaves, stems and roots were separated at each harvest; the stems and roots were then bulked for analytical treatment. The leaves and stems *plus* roots were weighed and then rapidly dried at

95° C. in an oven through which a rapid stream of air was passing; drying was practically complete in 10 min.

This technique was adopted in preference to the use of ground fresh material, since it permitted the determination of protein sulphur by oxidation in an Emerson calorimeter bomb as described by Barrien & Wood (1939). The bomb method cannot be applied to material containing the relatively large quantities of sand used in

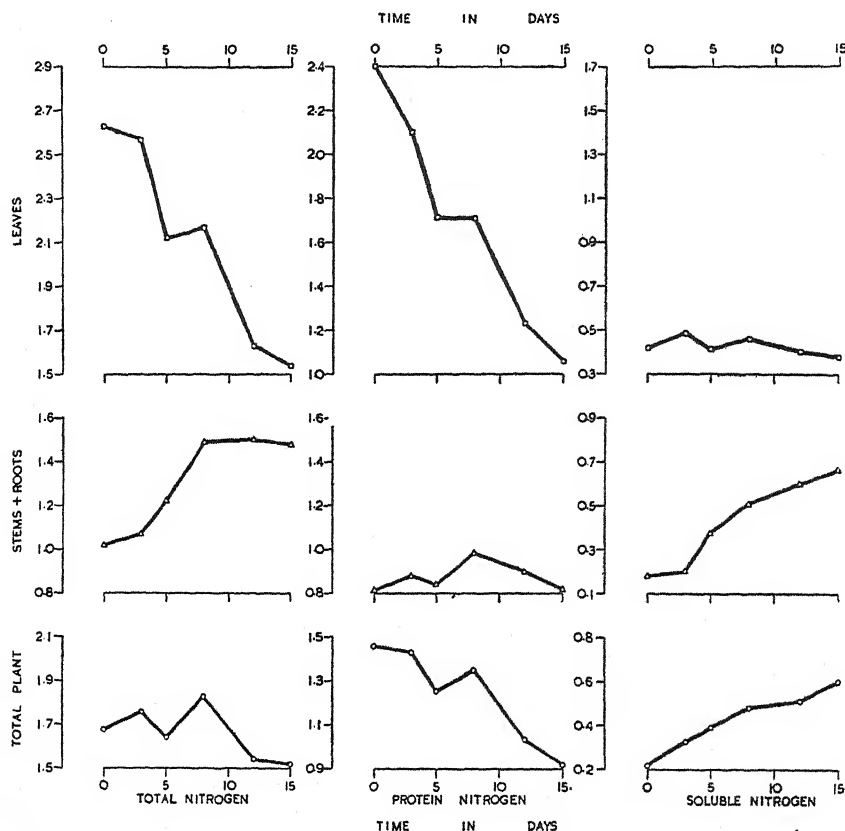


Fig. 3. Amounts of total nitrogen, protein nitrogen and soluble nitrogen compounds in grams per 100 g. dry matter in leaves, stems *plus* roots and total plant. From data of Table II, Exp. 2.

the grinding process. We have discarded the Benedict-Denis method, since it gives duplicates which agree to within only about 7 % and also because it gives a variable blank. Duplicate analyses by the bomb method agree to within 1 %.

The carefully ground dried material was prepared for analysis by extracting aliquots with tannin solution; inorganic and ethereal sulphates were determined in the filtrate and protein sulphur in the residue (Wood & Barrien, 1939). On replicate aliquots protein nitrogen was determined by the micro-Kjeldahl method according to Parnas-Pregl and subsequent iodometric titration. Total sulphur and total nitrogen were determined directly on the ground and dried material.

All analyses were performed on duplicate samples of the material and the mean value expressed on a dry weight basis. The results of analysis for leaves, stems *plus* roots, and for the total plant, together with dry weight data, are presented in Tables I and II and graphically in Figs. 1-3.

Table I. Results of Experiment 1

Dry weight in grams: all other fractions expressed in grams per 100 g. dry matter.

Day	Mean dry weight per pot	Water	Hexoses	Inorg. SO ₄ S	Inorg. + ethereal SO ₄ S	Cystine S	Prot. S
0	2.63	373	7.72	0.220	0.218	0.053	0.290
1	3.13	403	11.66	0.276	0.270	0.057	0.277
4	2.60	450	9.38	0.253	0.251	0.070	0.253
9	2.40	488	5.06	0.286	0.296	0.070	0.198
15	2.60	426	4.68	0.336	0.322	0.065	0.178

DISCUSSION

The results of Exp. 1 show that carbohydrates exert no "protein-sparing" effect, but that protein sulphur progressively decreases in amount from the beginning of the experiment and is accompanied by a progressive decrease in amount of hexoses and by an increase in the amount of sulphate sulphur. No ethereal sulphates could be detected in the leaves. The amount of free cystine increased on the fourth day but thereafter remained unchanged; it does not accumulate as the protein sulphur decreases.

It cannot be concluded from Exp. 1 that the loss of protein sulphur in the leaves is accounted for by ultimate oxidation to sulphate, since soluble sulphur compounds may have been translocated from the leaves or inorganic sulphate transported thereto.

However, in Exp. 2, the picture is similar to that in Exp. 1. In Exp. 2 the total sulphur content of the leaves did not change appreciably throughout the experiment except between days 0 and 3, where it is obvious that transference from the glass-house to the altered conditions of the dark room has resulted in translocation of sulphate sulphur from stems to leaves. The data for the whole plant, as well as for the leaves alone, indicate that decrease in amount of protein sulphur with time is accompanied by increase in amount of sulphate sulphur, and also that the whole of the loss in protein sulphur is accounted for by its oxidation to inorganic sulphate sulphur. Soluble organic sulphur compounds (presumably cystine, methionine and glutathione) as measured by the difference between total sulphur and protein *plus* sulphate sulphur remained approximately constant in amount.

Furthermore, it is clear that the hydrolytic and oxidative processes in sulphur metabolism were confined to the leaves and that the resultant product accumulated therein, for the amounts of protein sulphur and inorganic sulphate sulphur in the stems *plus* roots did not alter appreciably throughout the experiment.

This state of affairs differs in some respects from that which occurs in nitrogen metabolism under the same conditions. The data of Table II show that the amount

Table II. Results of Experiment 2

Dry weight in grams: all other fractions expressed in grams per 100 g. dry matter

	Day	Mean dry weight per pot	Water	Prot. N	Sol. N	Total N	Prot. S	Inorg. SO ₄ S	Total S	Ratio Prot. N Prot. S
(1) Leaves	0	4.84	614	2.40	0.42	2.63	0.183	0.435	0.638	13.1
	3	4.36	670	2.10	0.49	2.57	0.168	0.535	0.741	12.5
	5	4.78	703	1.71	0.41	2.12	0.124	0.540	0.663	13.8
	8	4.85	611	1.71	0.46	2.17	0.123	0.557	0.719	13.9
	12	3.73	564	1.23	0.40	1.63	0.095	0.625	0.742	13.0
(2) Stems plus roots	15	3.60	439	1.06	0.38	1.54	0.072	0.668	0.739	14.7
	0	6.79	n.d.	0.81	0.18	1.02	0.058	0.264	0.320	14.0
	3	5.36	n.d.	0.88	0.20	1.07	0.062	0.168	0.242	14.2
	5	5.44	n.d.	0.84	0.38	1.22	0.060	0.200	0.259	14.0
	8	4.45	n.d.	0.98	0.51	1.49	0.066	0.205	0.274	14.8
(3) Total plant	12	5.75	n.d.	0.90	0.60	1.50	0.060	0.205	0.270	14.9
	15	5.45	n.d.	0.82	0.66	1.48	0.059	0.195	0.272	13.9
	0	11.63	n.d.	1.46	0.22	1.68	0.110	0.339	0.452	—
	3	9.62	n.d.	1.43	0.33	1.76	0.111	0.336	0.471	—
	5	10.22	n.d.	1.25	0.39	1.64	0.094	0.359	0.462	—
	8	9.39	n.d.	1.35	0.48	1.83	0.096	0.387	0.489	—
	12	9.48	n.d.	1.03	0.51	1.54	0.074	0.370	0.452	—
	15	9.05	n.d.	0.92	0.60	1.52	0.063	0.382	0.458	—

of protein nitrogen remained approximately constant in the stems *plus* roots and decreased in the leaves in a way paralleled by protein sulphur. But whereas the sulphate produced ultimately from the sulphur in the protein molecule accumulated in the leaves, the soluble nitrogen compounds produced in hydrolysis (presumably chiefly amino acids and amides) remain approximately constant in amount in the leaves but accumulate in the stems and roots.

Both Mothes (1931) and Yemm (1937) found that in detached leaves kept in the dark the amount of protein nitrogen decreased whilst the amino acids and amides increased. Petrie & Wood (1938 *a, b*), in their experiments on nitrogen metabolism of plants under constant environmental conditions in the light, found that when amounts of protein were hydrolysed between any two days then the amino acids never accumulated in the leaves; they presumed that translocation was sufficiently rapid to keep pace with any appreciable amount of soluble nitrogen compounds formed above that characterizing the steady state in the leaves. It is possible that a similar mechanism is operative here.

Wood & Barrien (1939) have shown that at the steady state the total sulphate sulphur is unrelated to the organic sulphur fractions. The accumulation of sulphate sulphur with decreasing protein sulphur in the leaves is in agreement with the data of that paper. The high concentration of sulphate in the leaves is probably accounted for in part by the absence of any sink such as would be present in active growing points in the light.

In Exp. 2 part of the decrease in protein nitrogen in the leaves might be attributed to the accompanying decrease in amount of water (e.g. on days 12 and 15), for Petrie & Wood (1938 *a, b*) have shown that at a steady state the amount of protein is determined both by the amino-acid content and by the water content. But superimposed upon this is the effect of an additional factor operative under starvation conditions, for it is clear that in Exp. 2 loss of protein may occur in the leaves whilst the water content increases in amount; also in Exp. 1 the water content does not vary greatly but the amount of protein decreases with time.

A noteworthy feature in Exp. 2 is the fact that the value of the ratio of protein nitrogen to protein sulphur remains approximately constant throughout the course of the experiment in both leaves and stems *plus* roots. This result is different from that which occurs when the protein decreases during the life cycle of the grass *Andropogon sudanensis* Leppan & Bosman (Barrien & Wood, 1939). In the latter case it was suggested that the decrease in the value of the protein nitrogen/protein sulphur ratio during the ontogeny of the plant might be explained by the more rapid utilization of a labile protein relatively poor in protein sulphur.

A similar decrease in the value of the ratio might have been expected during starvation had the leaves of *Lolium subulatum* contained a similar protein. It is possible that more than one protein may be present in these leaves, but are hydrolysed to the same extent in the dark: it is also possible that biochemical differences may exist between plant species, and that in *Lolium* several proteins may occur each with similar contents of sulphur.

SUMMARY

Experiments are described wherein plants of the grasses *Lolium multiflorum* Lam. and *Lolium subulatum* Vis. were subjected to starvation conditions in a dark room at 16° C. and harvests made at intervals over a period of 15 days. Analyses were made of the contents of total sulphur, protein sulphur, inorganic sulphate sulphur, ethereal sulphate sulphur, cystine sulphur, total nitrogen, protein nitrogen, soluble nitrogen compounds, water and hexoses.

During the experimental period the protein sulphur decreased in amount in the leaves and was accompanied by a corresponding increase in sulphate sulphur. No ethereal sulphate could be detected and the amount of soluble organic sulphur did not alter appreciably. In the leaves protein nitrogen decreased in amount, but was not accompanied by an increase in amount of soluble nitrogen compounds. In the stems *plus* roots the amounts of protein sulphur, protein nitrogen and sulphate sulphur did not change appreciably during the experiment, but the soluble nitrogenous compounds increased in amount owing to translocation from the leaves.

The value of the ratio of protein nitrogen to protein sulphur did not alter appreciably in either leaves or stems *plus* roots.

It is concluded that during protein katabolism in leaves protein sulphur is oxidized, probably by way of cystine, to inorganic sulphate sulphur.

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THE DEFENSIVE MECHANISM IN ORCHID MYCORRHIZA

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(With Plate III and 1 figure in the text)

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INTRODUCTION

IN a discussion on the significance of mycorrhiza (1936) the writer expressed the view that the association between fungi and the roots of higher plants should be regarded as one of controlled parasitism. Such a view is by no means new. As early as 1904 Bernard suggested that the orchids were "plantes atteintes d'une maladie parasitaire chronique qui commence à la germination et persiste en général jusqu'à l'état adulte; maladie benigne en un certain sens, puisqu'elle n'empêche pas la vie, mais qui ne constitue pas moins une tare physiologique des Orchidées en général." It is the opinion of the writer that Bernard's remarks may be extended to all endotrophic forms. If such a view is correct, an understanding of the mechanism controlling the invading fungus is of interest, not only in respect to mycorrhiza, but also to plant disease in general. While it is desirable that all types of mycorrhiza should be investigated, any examination of the relationship between host and fungus in roots infected by the arbuscular fungus is difficult, since isolation of this type of endophyte, even when possible, is not easy. With orchid mycorrhiza no such difficulty arises. The following study has been undertaken in the hope that a more detailed analysis of the host parasite relationship in orchid roots may lead to a better understanding of root infection in general.

Numerous accounts of fungal infection in orchids are available, and the general outlines of the changes involved are well described. It is not known how the fungus is maintained outside the orchid plant, although it is generally assumed that the fungus is capable of existing in the soil, either as sclerotia or as active mycelium. However, it is clear that the invading fungus enters from the soil, usually through root hairs, or, less commonly, by direct penetration of the epidermis. Coils of

hyphae are formed in most of the cells of the middle cortex, and the nuclei of the host cells undergo marked changes (cf. Pl. III, figs. 3, 4). A general disorganization of the hyphae then follows, till finally only a structureless mass remains. Such a change in relationship between an invading fungus and its host is rare. During the first phase the fungus is an active parasite, invading living tissue; during the second, the parasitic activity is lost, and the mycelium already formed is destroyed.

It is difficult to estimate the speed at which these changes occur with any degree of accuracy. In *Orchis incarnata* L. the new roots usually become infected about April and by the end of May there is usually some evidence of breakdown. After the breakdown of the fungus a further invasion may often take place and in one plant examined there was evidence in some cells of three or four successive invasions. It seems that the changes in the hyphae take place more rapidly in second invasions and the examination of plants at frequent intervals suggested that in the secondary invasions there may be only 3 to 4 weeks between invasion and breakdown.

Despite the extensiveness of the root infection, orchid tubers are rarely affected. More seldom still is the fungus found in the stem or leaves. Bernard was working on this problem at the time of his death, and the results of his work are recorded in a posthumous paper (1911) on the fungicidal action of orchid tubers. He was able to show that a toxic substance, easily diffusible and destroyed by heat, was present in the living tissue. The substance, though toxic for various forms of the endophyte, did not affect other root fungi such as *Rhizoctonia Solani*. Later this work was confirmed by Nobécourt (1923) and Magrou (1924). The resistance of stem, leaves, tubers and roots to invasion suggests that the same mechanism may be present in each though to a lesser degree in the roots.

During the invasion of the roots it is possible to distinguish two phases in the resistance, a first phase concerned with mechanical penetration and subsequent wall-thickenings and a second phase in which active breakdown of the hyphae occurs. It is usual to regard the type of resistance found in the first phase as mechanical resistance. In the second phase the type of resistance is more difficult to designate, but it should probably be regarded as a form of protoplasmic resistance.

MECHANICAL RESISTANCE

Previous workers have shown that infection is frequently through root hairs, and this is undoubtedly true in many of the species examined by the writer, but not in all. Many preparations have been seen showing the hyphae penetrating directly through the epidermis. In *Gymnadenia conopsea* Br. this seems to be the rule and penetration through the root hair the exception. Usually, where the hyphae penetrate, the wall of the host remains unchanged. In certain orchids, however, e.g. in *G. conopsea*, marked changes have been observed. When plants of this orchid are removed from the soil it is often difficult to wash the roots free from decaying plant debris. Sections show large numbers of hyphae coming from the plant debris and penetrating the cells (Pl. III, fig. 1). The epidermis of the host is greatly modified in the vicinity of the penetrating hyphae. Beneath the masses of plant debris the walls are changed remarkably. They become somewhat distorted by pressure and

are often forced apart by the fungal hyphae which grow out from the adhering debris and pass between the cells. The walls become thickened and appear yellowish in colour. Frequently one or two cortical cells in the neighbourhood become similarly affected. When this happens, the general appearance is often that of a small lesion. The chemical nature of this wall thickening has not been investigated. In unstained sections it is yellowish and translucent; in stained sections it colours in the same way as the suberin of the endodermis, but not so strongly. It is probably some form of excessive cuticularization. The cortical cells adjacent to the hyphae show similar, but never such pronounced thickenings. In badly affected cells the layer of thickening may be 6–7 μ thick. Some preparations suggest that a similar suberin-like material is laid down around the penetrating hyphae, giving them a yellowish appearance. In general, throughout all mycorrhizal types, the hyphae in the epidermis and outer cortex frequently have yellowish walls. It seems that such hyphae are frequently not alive and are partially covered by a suberized layer. At times a deposition of suberin seems to prevent further growth of an invading hypha (cf. Pl. III, fig. 1). In many examples of fungal parasitism development of cellulose leads to the formation of collars of material around the hyphae at the point of entry. Burgeff (1936) and others have figured thickenings of this sort in *Gastrodia* and other species, but the writer did not observe them in the species he studied.

PROTOPLASMIC RESISTANCE

(a) *Histological changes in the hyphae*

The mechanical resistance described above is relatively unimportant when compared with the breakdown of the invading hyphae in the inner cortex. This breakdown, although recognized for many years, has never been adequately studied, and apart from description of changes in the general morphology of the endophyte, there is little precise information about the changes which occur in the hyphae during the early stages of digestion.

The fate of the endophytic fungus has been a question of much interest. Magnus (1900) described the presence of two types of host cells, the *Pilzwirthezellen* and the *Verdauungszellen*, but the separation seems to depend on the outcome of the interaction of the host cells and the fungus, rather than on any inherent differences in the cells themselves. The early stages of the digestion of the hyphae are accompanied by a most striking change in the staining capacity of the fungal cytoplasm. During infection of the cells the hyphae are filled with protoplasm and usually contain granules of glycogen and other materials. Following any of the usual procedures, it is easy to stain the nuclei and cytoplasm and obtain good preparations. At the beginning of the digestion it becomes increasingly difficult to destain the hyphae, as they become extremely retentive of stains (cf. Pl. III, fig. 2). During the early stages of this change from normal hyphae to *Eiweiss-hyphen*, as they were designated by Magnus, the diameter of the hyphae increases slightly and the vacuoles, which first increase in number, disappear. In living material the colour changes from the typical faint blue-grey of living protoplasm to a translucent oily yellow in which detail is lost. The normal fungal nuclei are small and spherical, with a strongly staining

nucleolus. As the changes in the cytoplasm take place, the nuclei shrink a little and become pyknotic and irregular in outline.

The staining reactions of the *Eiweisshyphen* have often been interpreted as being indicative of a great increase in protein material in the hyphae. Similar changes in staining reactions have been observed in hyphae subjected to the extracts from orchid roots (cf. p. 279). There seems little doubt that these changes are to be correlated with the degeneration of the cytoplasm to give initial breakdown products which are more retentive of stains than the original cytoplasm. The breakdown of the protoplasm continues until finally the hyphae become almost empty (cf. Pl. III, fig. 3). At this stage the degenerate pyknotic nuclei are still present. The walls of the hyphae then crumple and collapse. Eventually the hyphae collect together in the centre of the cell and form the yellowish mass variously called *corps dégénération*, *corps jaunes* etc. (Pl. III, fig. 4). Little is known of the changes which the hyphal remains undergo. In the final stages the residue is more or less homogeneous and appears to resist complete breakdown. The *corps jaunes* are often recognizable in roots which have almost completely decayed.

(b) *Vitality of the endophytic mycelium*

At some stage during the changes described above the fungus loses its vitality. Some hyphae, for example those undergoing digestion, have obviously lost their capacity for further growth. Others, usually in the outer cortex of a newly infected root, are quite active. Between these two extremes there are numerous stages in which the hyphae may or may not be active. One criterion of life often referred to during the examination of a plant cell is its ability to be plasmolysed. It is usually considered that if a cell can be plasmolysed it is still alive. When examining parenchyma cells it is easy to determine whether they are plasmolysed. However, the examination of fungi in this way is by no means easy, particularly when the hyphae are narrow or filled with abundant protoplasm. Furthermore, the osmotic pressure of the hyphal contents is so much greater than that of the cells of the orchid root, that solutions which are likely to cause plasmolysis of the hyphae cause violent contraction of the orchid cells. This leads to a clumping of the hyphae which renders subsequent microscopic examination almost impossible. Direct plasmolysis tests were seldom made; it was found better first to dissect out the hyphae and then to examine them. Another method of determining the vitality of the hyphae at any particular stage is to transfer them to nutrient agar and see whether they are still capable of growth. Both methods have been used in the present investigation.

Plants of *Orchis incarnata* were brought in from the field and adhering soil was washed from the roots. After a preliminary examination, suitable pieces of root, free from deformities and external injuries, were chosen and cut into pieces about 1-2 cm. long. These were placed in mercuric chloride (1/1000) for 2 min., washed in sterile water and allowed to remain damp in a sterile Petri dish for 6-8 hr. They were then placed in saturated chloride of lime for 5 min. and again washed. Such careful sterilization tends to injure the hyphae in the outer part of the root. Accordingly hyphae from the outer layers were examined without

sterilization. This naturally led to considerable contamination. To reduce this, plain agar was used, and each isolation was treated with a drop of sterile 5 % lactic acid to discourage bacterial growth. Tests with and without lactic acid showed that the acid had no marked inhibitory action on the growth of the hyphae.

A Janse-Péterfi micromanipulator was used for dissecting the hyphae from the cells. Hand sections about one and a half cells thick were cut from a root, with a flamed razor, and mounted in a drop of sterile water on a coverslip in the damp chamber of the dissecting apparatus. The coverslips used for the purpose were kept in alcohol and flamed before use. Water was withdrawn from the drop with a pipette till the section adhered firmly to the glass. A suitable mass of hyphae was then chosen, placed in the centre of the field and two stout needles introduced. The cell was torn, and any pieces of hyphae adhering to the wall were broken. A pipette with an opening slightly larger than the hyphal clump was introduced and the hyphae were withdrawn. The pipette was removed and the contents expelled on to a clean part of the coverslip. Sterile water was added from a second pipette and withdrawn with the first. This washing was repeated four times and helped to reduce contamination. Finally, the hyphae were transferred to agar with another sterile pipette. The agar was filtered before use to facilitate microscopical examination. If the hyphae were still active, some growth was visible after 24-48 hr., and even when the plates were contaminated, the contaminant was seldom advanced enough at that stage to prevent observation. In addition to the isolation of hyphae from the interior of the root, some of the hyphae from the root surface were dissected off and examined. Other similar hyphae were isolated in this manner and then tested to see whether they could be plasmolysed. After they were removed they were transferred to drops of a strong solution of cane sugar. The results of these experiments are given in Table I.

Table I. *Vitality of mycelium in the roots of Orchis incarnata*

(For a fuller description of this table see p. 278)

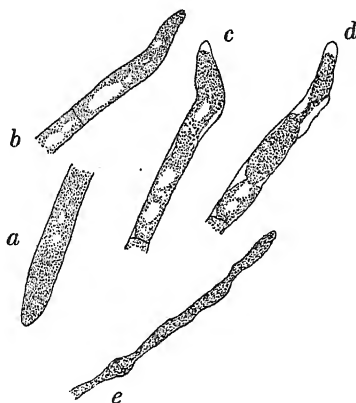
Type of hyphae	No. of isolations	No. showing growth	No. of isolations	No. plas-molysed
Brownish hyphae from surface of root. Probably the endophyte	5	3	4	3
Brownish hyphae from the surface of root, with clamp connexions. Not the endophyte	6	6	3	2
Hyphae from outer cortex in young infected root	9	9	3	3
Hyphae from outer cortex in older root. Walls slightly yellow. Hyphae in inner cortex digested	5	2	16	5
Hyphae from central cortex, no apparent abnormality. No digestion showing in section	4	4	2	2
Hyphae from central cortex, showing slight swelling. Some cells showing early stages of digestion	8	1	9	4
Typical <i>Eiweisshyphen</i>	7	0	10	4
Apparently empty hyphae beginning to crumple	14	0	8	1
	5	0	—	—

Columns 4 and 5 contain the results of the experiments to test the vitality of the hyphae, using plasmolysis as an indicator of vitality. Often it is difficult to be certain whether or not plasmolysis had occurred; for example, in one experiment, of nine pieces of hyphae examined four were plasmolysed and three were not. The remaining pieces showed a slight crumpling of the walls which might have been incipient plasmolysis or might have been due to mechanical injury. Unless definite plasmolysis was apparent, the hyphae were considered as not plasmolysed. The results show that during the early stages of infection the hyphae are vigorous and capable of further growth. During the early stages of filling of the cortical cells they are still active. However, once any change is visible, their activity is very much less. In view of the results obtained with the hyphae showing slight swelling, some of the material was stained, using gentian violet and iodine. Hyphae at this stage show little trace of abnormality in their staining reactions. The only difference observed from the normal hyphae was an increase in the number of vacuoles. There was no evidence of the staining typical of *Eiweisshyphen*. Although only one out of eight pieces grew, four out of nine could be plasmolysed. In a repetition of the experiment a similar result was obtained. Another interesting feature is the poor growth of hyphae from the outer cortex of older roots. These hyphae showed no signs of digestion, nor did they appear abnormal apart from the yellowish colour of their walls. Some of them were typical of the clumps found in the *Pilzwirthezellen*. In many such hyphae in fixed preparations it is difficult to demonstrate protoplasmic contents. In the past the writer has regarded this as due to faulty technique rather than to an absence of contents, but in view of the inability of some of these hyphae to grow, this opinion may need revision.

(c) *Fungicidal action of orchid extracts*

In order to examine the disintegration of the hyphae more fully, extracts were made from the tubers, stems, leaves and roots in the following manner. The tissue was chopped into small pieces and some distilled water was added. The mixture was frozen, allowed to thaw, and then squeezed through muslin, the final pressure being applied by placing the bag between two stainless steel disks held in the jaws of a bench vice. In some experiments the resulting fluid was used without further treatment, but in most it was centrifuged for 20 min. and then decanted from the precipitate; in others an excess of chloroform or alcohol was added and the precipitate removed after 2 days. The precipitation methods however were found to be unsatisfactory. In some experiments 5 or 10% glycerin was used as an extracting medium. Extracts prepared in this way apparently contained more proteolytic enzymes than aqueous extracts, and hyphae subjected to these extracts showed a much more rapid breakdown of the cytoplasm. All extracts contained considerable amounts of mucilage and proteins. Attempts to remove these materials without greatly reducing the activity of the preparations have met with little success. The activity of the extracts was tested by adding small quantities of the extracts to young cultures of the endophyte growing on agar smears. The agar smears were prepared as follows: coverslips were placed in alcohol for some time and then flamed; one side

was painted with hot 5 % malt extract agar so that a fairly thin film was formed. The smears were then inoculated with the endophyte and mounted as in the usual hanging drop method. It was found most convenient to place the inoculum at one edge of the smear rather than at the centre. At the end of 3 or 4 days the fungus had grown over about half the agar. The fungal culture used was one of the many obtained from dissected hyphae. A drop of the extract to be tested was then placed on the agar next to the advancing edge of the fungal mycelium. The following are the detailed observations of one experiment, using an extract from the tubers prepared with 5 % glycerin (cf. Text-fig. 1).



Text-fig. 1. *a-e*, successive stages in the collapse of a hypha under the action of the toxic principle from the orchid tuber: *a*, when the extract was added; *b*, 7 hr. later; *c*, after 22 hr.; *d*, after 2 days; *e*, after 4 days.

- 23 July. Agar smears prepared, and inoculated.
- 26 July. Extracts from the tubers prepared.
 - 11 a.m. All hyphal tips filled with dense cytoplasm, a few vacuoles about 20-30 μ from the tips. Septa remote from the tips.
 - 11.15 a.m. Extract added to each coverslip. The drops were gradually absorbed by the agar.
 - 6.30 p.m. Hyphal tips nearest the place where the extract was added slightly deformed. Cytoplasm more vacuolate, some hyphae with a septum formed close to the tip.
- 27 July. 9.30 a.m. Most hyphae shrivelled to some extent, cytoplasm more vacuolate, tending to shrink away from the walls.
- 30 July. Most hyphae completely shrivelled, the cytoplasm more or less homogeneous, oily yellow. Experiment discontinued.

In control experiments with boiled extracts the growth of the hyphae was normal and vigorous. When different extracts were used, the results seemed similar and such differences as were recorded seemed to be related to the strength of the extract. Extracts prepared from the roots were much less active than similar ones prepared from the tubers or stems.

In order to show beyond doubt the presence of a fungicidal substance in the host cells in which digestion was proceeding, the following technique was adopted. Using methods similar to those described on p. 277, a small pipette, with a mouth about $5-10\mu$ in diameter, was inserted into a cell and the sap withdrawn. The success of this operation could be judged by observing the protoplast of the cell. In most infected cells the protoplast can be seen in the unstained material. When the cell sap is withdrawn, the protoplast collapses and an air bubble fills the cell. The sap was collected from about 30 to 100 cells and then expelled on to a dry part of the coverslip and allowed to dry. It was redissolved in a very much smaller quantity of water, and the solution was then ejected on to an agar smear culture. In the early experiments the results were irregular. One reason seemed to be that the extracts rapidly diffused through the relatively large volume of agar and became too dilute. To prevent this a small area of the culture, slightly less than 1 mm. square, was selected and the agar surrounding this removed with a sterile razor-blade. Such small pieces of agar dried out rapidly, even though placed above a drop of water. The only way found to avoid this drying was to coat the inside of the welled slide with fine droplets of water. A small quantity of water was placed in a welled slide and the coverslip sealed down with wax. The slides were put in an incubator at 35°C . for half an hour and then placed on a sheet of cold metal. Usually this caused a fine deposit of dew over the inside of the slide. The coverslip was then replaced by the one bearing the agar with the fungus and the extract. In this way it was found possible to keep small pieces of agar for at least 2 weeks without any trace of drying appearing.

In the latter experiments many sections were used and sap was collected from a large number of cells. Usually a day was spent in collecting the sap, which, after being concentrated, was placed on one of the small blocks of agar. Twenty-four hours later visible changes appeared, and at the end of 4 days some empty hyphae could be seen. A week after the extract had been added to the hyphae the agar blocks were transferred to agar plates. Control pieces of the smear culture showed no trace of any breakdown and gave active cultures when transferred to agar plates.

Apart from their fungicidal action, the only property of the extracts so far examined has been their reaction to heat. In this regard the original observation of Bernard (1911) has been confirmed. Extracts heated for 5 min. at 50°C . were active, those heated for a similar time at 60°C . were deactivated. The extracts deactivated in this way produced no visible effects when placed in contact with the living endophyte.

The protoplasmic resistance described above is apparently widespread throughout the plant, although it is marked in the stems and leaves. An interesting observation has been made regarding the relation between chlorophyll formation and resistance to infection, and although it does not strictly belong to the present section of the discussion it may well be mentioned here. In the aerial roots of epiphytic orchids, e.g. *Sarcochylas*, the sides of the roots away from the leaves or branches on which the orchid is growing often develop chlorophyll in the cortical cells. Such roots are infected on only one side. The formation of chlorophyll seems to be

correlated with an immunity to infection in the cells. A similar immunity has been produced artificially by the writer in the roots of *Orchis incarnata*. During the period of active root growth in the early spring, and before a great deal of infection had taken place, the soil was removed from some of the plants so that the upper surfaces of the roots were exposed to the light. After about 2 weeks the roots were a decided green. Six weeks after they had been uncovered the roots were dug up and examined. Cross sections of these roots revealed that the cells on the upper side possessed chloroplasts and showed no trace of infection, while those on the lower side were typically infected. Thus, clearly, development of chlorophyll was accompanied by an immunity from infection.

DISCUSSION

The breakdown of the hyphae in the root is probably the most striking phenomenon associated with mycorrhizal infection and is responsible to a large extent for the separation of mycorrhizal infection from the more usual forms of parasitic invasion. It is natural to expect that the breakdown is largely enzymatic and several workers in the past have attempted to examine the enzymes present in a mycorrhizal system. Shibata (1902), for example, was able to show that there were proteolytic enzymes in the roots of *Podocarpus*. More recently, Fuchs & Ziegenspeck (1924) examined *Orchis Traunsteineri* and reached the conclusion that there are, in the root, numerous enzymes including amylases and peptic and peptolytic enzymes in addition to enzyme systems which can break down substances in the humus. In the roots of *Orchis incarnata* there is little doubt that peptolytic enzymes are present, and it seems probable that the breakdown of the fungus protoplasm is due to enzymes produced by the root. Muller (1936) considers that, in *Citrus*, part of the fungal breakdown is due to autolysis. Whether autolytic breakdown is wide-spread in mycorrhiza is not known, but it is quite possible that it may play a more important part than is generally recognized.

Bernard (1911) reached the conclusion that the fungicidal material in the orchid tubers was an antibody secreted by the cells of the orchid under the influence of an antigen produced by the fungus. Nobécourt (1923), during a re-examination of the problem, found that not only was the supposed antibody absent from tubers which had been heated or subjected to chloroform but also from tubers which had been frozen to -15°C . He concluded, however, that the lack of fungicidal properties in the tubers which had been frozen and then placed in contact with the fungus was not due to the destruction of the material already there, but that the tuber tissue had been killed and was thus unable to produce the antibody. Magrou (1924) did not agree with this interpretation and he was able to show that the fungicidal material was present before the fungus had come in contact with the tubers.

During the present work it has been shown clearly that there is a complete breakdown of the fungal cytoplasm and there is no doubt that proteolytic enzymes are present. However, there is no evidence either for or against the existence of antibodies. The results of Bernard, Magrou and Nobécourt may well be interpreted on the grounds that a thermolabile toxin is present. The substance need not

necessarily be of the nature of an antibody, and indeed, until there is proof of the existence of an antibody in orchid roots, the use of such a term seems premature, particularly in view of its specialized meaning in bacteriology.

During the breakdown of the fungal material quite large amounts of fungal protein are liberated into the cells of the root. This may represent a gain in organic material for the higher plant or, on the other hand, it may only be the return of material taken from the plant during the parasitic phase. Any discussion on this aspect of the problem, however, is beyond the scope of the present paper.

SUMMARY

1. In orchids fungal infection of a mycorrhizal type is usually limited to the roots. Tubers, stems and leaves are not normally infected.
2. Two types of resistance are recognized, a mechanical resistance in the form of wall thickenings and cuticularization and a protoplasmic resistance. In the latter there is a complete breakdown of the invading fungus.
3. During the breakdown of the fungus, histological changes can be observed; these have been correlated with a loss of vitality of the endophyte.
4. The vitality of the endophyte at different stages has been tested by dissecting out the hyphae by means of a micromanipulator and transferring the hyphae to agar. Plasmolysis experiments have also been used.
5. Bernard's observation that a toxic material exists in the tubers has been confirmed.
6. Using micropipettes, a toxic material has been isolated from the cells in which the fungus is undergoing digestion.

ACKNOWLEDGEMENTS

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Fig. 1.



Fig 2.

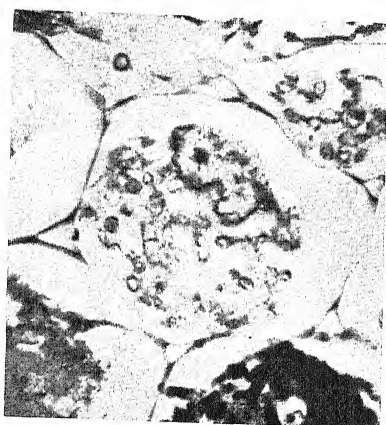


Fig. 3.



Fig. 4.

EXPLANATION OF PLATE III

Fig. 1. *Gymnadenia conopsea*, showing an attached mass of decaying debris. Most of the fungal hyphae which have penetrated have become enveloped in some form of suberin which has stopped their growth. The epidermal cells show pronounced thickening of their walls, which are sometimes forced apart. $\times 450$.

Fig. 2. *Orchis mascula*, a cortical cell from the root, showing the densely staining *Eiweisshyphen*. $\times 360$.

Fig. 3. *Orchis mascula*, showing the empty hyphae after the protoplasm has been broken down. Figs. 2 and 3 are both from the same root section. $\times 360$.

Fig. 4. *Orchis mascula*: *a*, the disintegrated mass of hyphae (*corps dégénération*); *b*, hypertrophied nuclei showing chromatopyknosis; *c*, a normal nucleus in an uninfected cell. $\times 360$.

MECHANICAL STIMULATION AND RESPIRATION IN THE GREEN LEAF

II. INVESTIGATIONS ON A NUMBER OF ANGIOSPERMIC SPECIES

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(With 2 figures in the text)

INTRODUCTION

IN a previous paper (Audus, 1935) a description was given of experiments in which the laminae of isolated leaves of cherry-laurel were carefully rubbed and bent at intervals during starvation in the dark. The effect of this relatively gentle treatment on the starvation respiration was a sudden and very considerable augmentation of the rate of CO_2 evolution of the leaves after each stimulation, and this heightened activity gradually subsided over a period of 2-3 days. The striking nature of this phenomenon and its bearing on experimental technique involving the handling of non-rigid tissues such as leaves indicated the desirability of investigations on species other than cherry-laurel. Experiments have therefore been carried out on leaves of a number of species of plants showing a considerable range of morphological leaf types, and chosen at random from those available within easy reach of the laboratory.

EXPERIMENTAL DETAILS

The whole procedure for studying the respiration drifts of these isolated leaf samples at 22.5°C . was essentially the same as in the experiments previously described (Audus, 1935). As will be seen from the graphs of Figs. 1 and 2, drifts of all the leaf samples show main features similar to those shown by the drifts of cherry-laurel leaves. One or two points should be noted. First, the duration of the protoplasmic respiration varies in the species chosen. In the evergreen xeromorphic species (*Laurus*, *Hedera*, *Prunus*, *Rhododendron*, *Yucca*) it resembles that of cherry-laurel in showing a long almost level phase lasting for a period of several days before the onset of senescence. In the mesomorphic species it is of shorter duration, and in *Bambusa* and *Pelargonium* the senescent phase immediately succeeds the floating respiration phase. A second difference between the respiration drifts of the evergreen xeromorphic species and the mesomorphic species is that the general level of respiration rates in the various phases tends to be higher in the latter species. This is presumably due to the greater percentage of dead cell-wall material in the xeromorphic species, since the respiration rates are calculated on a basis of fresh

weights. It has been shown that in the cherry-laurel, successive stimulations carried out on the protoplasmic respiration phase resulted in the most uniform series of effects, and thus in these experiments stimulations were carried out as nearly as possible on the corresponding protoplasmic phases.

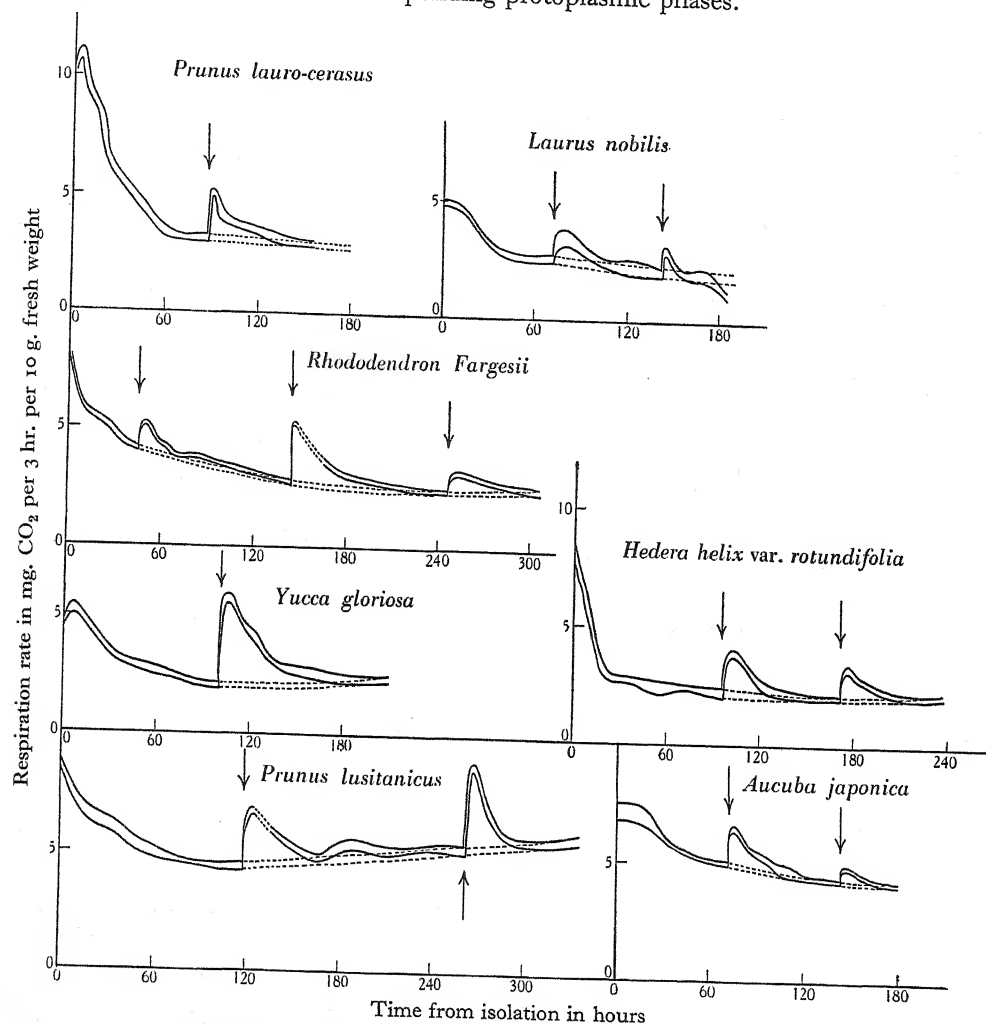


Fig. 1. Graphs showing the starvation respiration drifts at 22.5° C. and the effects of mechanical stimulation in the xeromorphic evergreen leaf types. The times of stimulation are marked by vertical arrows.

The range of different morphological types in the leaves studied made it necessary to modify the stimulation technique in some cases. With all the evergreen leaves and also those leaves having a long narrow blade (*Phragmites*, *Typha*, *Bambusa*, *Sparganium*, *Iris* and *Yucca*) the technique previously described for cherry-laurel was used. In the cases of the non-linear mesomorphic leaves (*Polygonum*, *Pelargonium*) attempts to carry out this type of stimulation resulted in a tearing of the leaf

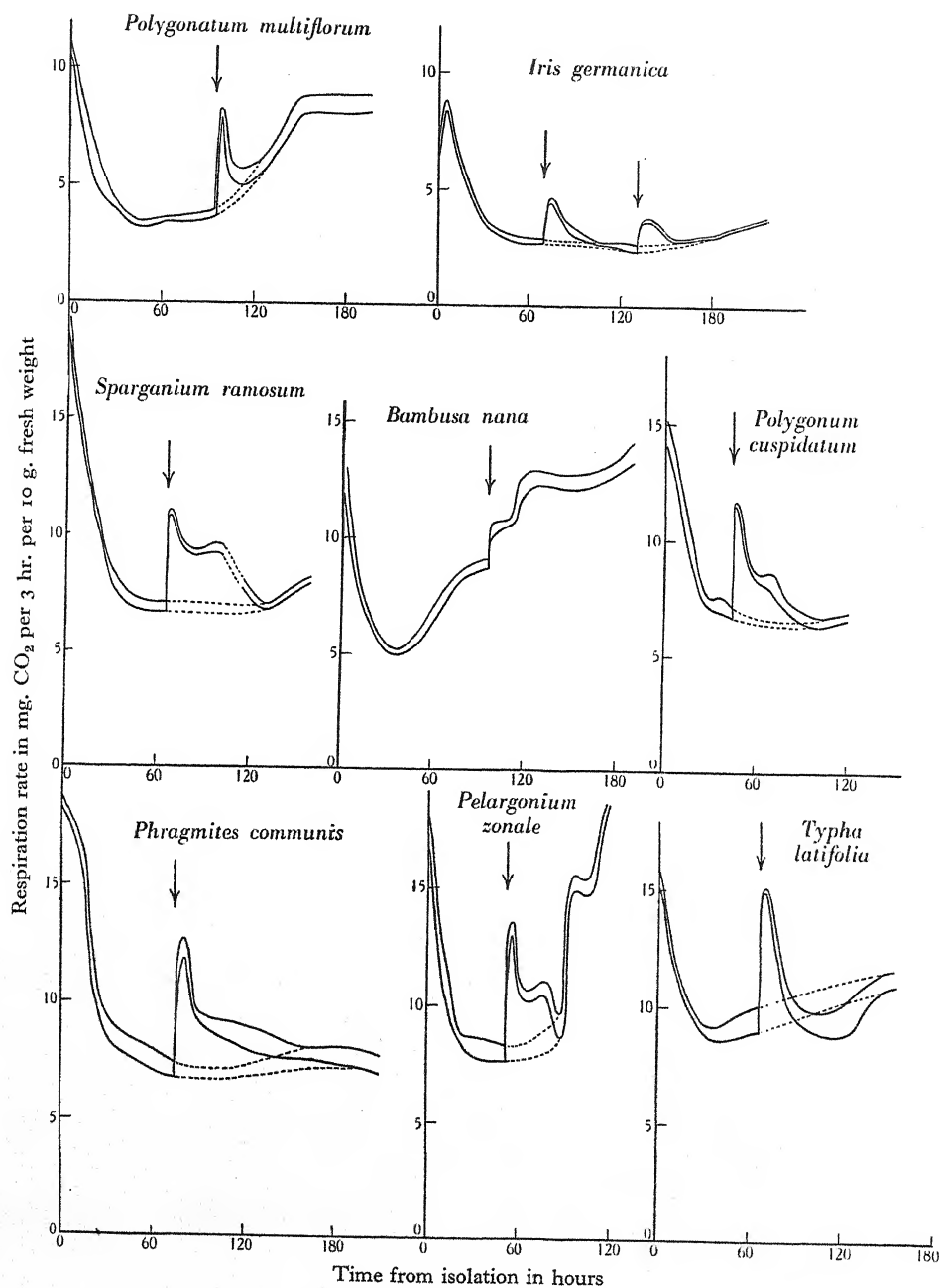


Fig. 2. Graphs showing the starvation respiration drifts at 22.5° C. and the effects of mechanical stimulation in the mesomorphic leaf types. The times of stimulation are marked by vertical arrows.

lamina. Stimulation of these species was therefore carried out by taking each leaf between the thumb and forefinger of each hand and gently bending the lamina in all directions. The leaf cells were not visibly damaged by this procedure.

RESULTS

The results of experiments on the leaves of fourteen species of plant are shown in Figs. 1 and 2, where the smoothed respiration drifts have been brought together for comparison of the stimulation effects. These curves are double, the two lines representing the limits of scatter of the individual respiration readings. Fig. 1 shows the curves obtained from the xeromorphic species with a curve from cherry-laurel for comparison. Fig. 2 shows the results for the mesomorphic leaf types. The smooth curves from actual observed readings are recorded in unbroken lines, while probable normal starvation drifts are interpolated as dotted lines. The times of stimulation are indicated by vertical arrows.

These curves show that, with the doubtful exception of the results from *Bambusa nana*, mechanical stimulation of all the species studied gave rise to respiratory responses which are strikingly similar in both form and duration, and are, as far as can be judged, identical with those described for cherry-laurel. Comparison of the results for the two leaf types shows that the stimulation rise (height of the initial peak of the effect above the normal drift level) in the mesomorphic types is on the average larger than in the xeromorphic types. These larger effects are correlated with the generally higher drift levels.

Table I

Species	Hours from isolation to stimulation	Percentage stimulation rise
Xeromorphic evergreen species:		
<i>Prunus lauro-cerasus</i>	87	63.0
<i>Rhododendron Fargesii</i>	45	26.0
	144	89.0
<i>Laurus nobilis</i>	246	34.3
	72	41.5
<i>Aucuba japonica</i>	142	42.4
	72	50.6
<i>Hedera helix</i> var. <i>rotundifolia</i>	143	29.1
	96	84.7
<i>Yucca gloriosa</i>	172	68.5
<i>Prunus lusitanicus</i>	100	182.8
	188	52.3
	262	66.1
Mesomorphic species:		
<i>Sparganium ramosum</i>	66	58.7
<i>Typha latifolia</i>	66	55.5
<i>Polygonum cuspidatum</i>	45	66.6
<i>Polygonatum multiflorum</i>	94	98.8
<i>Pelargonium zonale</i>	52	58.3
<i>Bambusa nana</i>	96	18.8
<i>Phragmites communis</i>	75	72.9
<i>Iris germanica</i>	69	52.3
	130	44.8

The values of the percentage stimulation rise

$$\left(\frac{\text{Stimulation rise}}{\text{Normal drift level at stimulation}} \times 100 \right)$$

have also been calculated for each effect and have been recorded in Table I. These figures show no systematic differences between the two leaf types, but, with one or two notable exceptions, are of the same order throughout the whole range of species. Of these *Yucca gloriosa*, with a percentage stimulation rise of 182.8, is the most outstanding exception. In *Bambusa nana* it is very difficult to estimate the percentage stimulation rise, but the low value taken (18.8 %) can be explained on the assumption that, as in cherry-laurel, senescence and leaf yellowing destroy the capacity of the cell to respond to stimulation. The leaves of this species were approximately 50 % yellowed when stimulated. It is interesting to note that the results for *Typha latifolia* suggest the occurrence of a secondary depressant effect similar to that occurring in the cherry-laurel under some conditions.

CONCLUSIONS

These few results suggest that the respiratory response induced by rubbing and bending of the leaf lamina is a widespread phenomenon in the leaves of angiosperms. The bearing of this respiratory sensitivity of the cell on experimental technique is obvious, and it would be instructive to know what part this phenomenon has played in the results of past investigation on respiration, where considerable handling of material was an integral part of the methods used.

REFERENCE

- AUDUS, L. J. (1935). Mechanical stimulation and respiration rate in the cherry-laurel. *New Phytol.* 34, 386.

REVIEW

Principles of Paleobotany. By W. C. DARRAH. A New Series of Plant Science Books, edited by F. VERDOORN, Vol. III. $6\frac{1}{4} \times 9\frac{3}{4}$ in. 239 pp., 6 pl. Leiden, Chronica Botanica Co.; London: Wm. Dawson and Sons, Ltd. 1939. 7 guilders or about 15s.

Dr Darrah of Harvard states in his Foreword that his purpose in this work has been to produce a general introductory survey of the field of paleobotany. This aim has been fully attained, and the list of references accompanying each chapter will give the reader every help in pursuing the subject further; but there is perhaps undue hopefulness in the author's remark that, since most of the cited papers are accompanied by comprehensive bibliographies, the reader "can soon cover the subject well". Throughout the book the geological aspect is kept steadily in view, and the account of the changes which the face of the earth has undergone is excellently dynamic. The author's correlations of American and European horizons, where these are important in fossil botany, will be useful on this side of the Atlantic.

In the introductory section, the history of paleobotany is briefly sketched; an unusual feature is the inclusion of the names and nationalities of those now at work upon the subject, as well as of those whose contribution is of the past. Another preliminary chapter, on paleobotanical techniques, gives full practical directions for the making of sections, nitrocellulose peels, coal macerations, etc. In the main body of the work, the different phyla of fossil plants are discussed, partly in botanical grouping, and partly as floras. The lively freshness of Dr Darrah's style carries the reader through the mass of detail presented, but it is questionable whether the student can be expected to master this vast array of facts, with only the limited aid of the relatively few and often over-reduced line drawings with which the book is illustrated; these are described as "plates", but in this country they would probably have been called text-figures. If, as is likely, Dr Darrah's book runs into further editions, it would be a great advantage if more pictures could be added, for illustrations are of paramount importance in a subject in which the student can never actually see or handle more than a very small proportion of the types which he is expected to visualize.

Dr Darrah bases his general views upon the telome theory, and boldly gives a phylogenetic chart deriving all the vascular plants from the Psilopsida-complex. He discusses with vigour and acuteness many of the problems with which paleobotanists are faced, but his conclusions occasionally bear the marks of over-rapid thought. This is noticeable, for instance, in his account of the relations of woody and herbaceous plants. After alluding to the anatomy of certain petrified woods, he remarks "that nothing contradictory to the notion that the woody habit is the oldest among angiosperms is found among these Cretaceous woods"; but the Cretaceous woods themselves can hardly be expected to produce evidence against this hypothesis. After reviewing the opinions of various authors, Dr Darrah finally accepts the idea that it "may be assumed with confidence that the woody habit is ancestral"; but it may be pointed out that H. A. Senn's recent discussion of the question (*Bibliographia Genetica*, 12, 1938, 303 *et seq.*) demonstrates the insecurity of this assumption.

Within less than 250 pages, Dr Darrah has indeed given the student of fossil plants overflowing measure, but yet it may be doubted whether the book, admirable as it is, quite fulfils the promise of its title. Paleobotany is relatively so young a science that the time is perhaps scarcely ripe for that organized and integrated examination of its modes of thought, which must precede any valid attempt to analyse them into their principles.

AGNES ARBER

NOTICE

Merton Catalogue of Chromosome Numbers

ATTENTION is drawn to the fact that copies of this catalogue, issued as *New Phytologist Reprint* No. 20, can be obtained at 2s. net from the Cambridge University Press, 200 Euston Road, London, N.W. 1.

The authors wish us to say that "people in a position to supply living material or seeds of plants whose chromosome numbers are missing from the Merton Catalogue are asked kindly to communicate with the Registrar of the John Innes Institution".

ERRATA

New Phytologist, Vol. 37, No. 1, 1938, p. 51

Line 4 from foot: *for* "stigmas as long as style" *read* "stigmas twice as long as style"

Line 2 from foot: *for* "stigmas half as long as style" *read* "stigmas as long as style"

SOCIETY FOR EXPERIMENTAL BIOLOGY

THIS Society holds three conferences each year at which papers and demonstrations are given on various aspects of experimental botany and zoology. Arrangements have been made whereby it is possible to obtain the New Phytologist through membership of the Society. Further particulars of membership can be obtained from the secretary, Prof. T. A. Bennet-Clark, University College, Nottingham.

The Society for Experimental Biology held a meeting at the gardens of The Royal Horticultural Society at Wisley on 10 July at which Prof. F. E. Weiss, F.R.S., presided.

A discussion on "Growth substances in relation to vegetative propagation" was opened by Dr Tincker. He pointed out that 3-indolyl-acetic, 3-indolyl-butyric, and α -naphthyl-acetic acids induce cell divisions in cambium and adjacent cells, and sometimes in epidermis cortex or pith. Effective concentrations lie between 10 and 100 p.p.m. Accelerated root production results in almost all readily propagated plants, but of the species listed by the Hormone Committee as "difficult" some 85 per cent have so far failed to respond. Water solutions and also mixtures with talc (1 part in 1000 or 5000) were effectively used. Additions of aneurin (40 p.p.m.) increased root growth only in certain cases.

Although deamination of tryptophane yields these active products, deamination of tyrosine and histidine yield products which are almost inactive. The activity of α -naphthyl-acetic acid is in contrast with the inactivity of the corresponding phenanthrene, phenenanthrene, and cyclopentylidene derivatives.

Dr F. L. Pyman, F.R.S., discussed the chemical nature of some of the more active root-promoting substances. The most active yet obtained (investigated by Dr Tincker) is a 1:2:3:4: tetrahydronaphthylidene acetic acid. Of the two (*cis* and *trans*) isomers the one with low m.p. (93°C.) is the more active. The mixture of these two acids with a dihydronaphthyl-acetic acid is available commercially and is more active than 3-indolyl-butyric and α -naphthyl-acetic acids which are the next most active compounds.

Dr Metcalfe pointed out the need of an extended anatomical survey and mentioned that certain anatomical features were generally associated with the absence of rooting of "difficult" cuttings. These fell into three main categories: plants with complete sclerenchymatous pericycles, those with very deep phellogen often cutting off the phloem, and those with secretory canals in the pericycle. Easily rooting cuttings do not possess these features. It is provisionally suggested that the first feature acts by offering a mechanical obstacle while the others may have nutritional or biochemical effects.

Mr Plant referred to the bud-inhibiting effects produced by these root-promoting substances, and showed that inverted pieces of sea-kale root could be induced to

form buds at the apex by artificial removal of hormone (cutting off series of slices) and roots at the base by supply of indolyl-acetic acid there.

Profs. Green, Gregory, Stoughton, Dr Ludford and Mr Moseley also took part in the discussion.

Mr Fox Wilson gave a paper on the "Bulb and stem eelworm *Anguillulina dipsaci* in relation to horticultural crops". The host range of this species of eelworm has been investigated, and a number of biologic races isolated infecting *Phlox*, *Narcissus*, *Hyacinthus*, *Iris*, *Oenothera*, *Scilla*, *Allium*, and potato. In *Phlox*, polyphagous races infecting *Phlox*, *Oenothera*, *Gilia*, *Collomia*, and *Primula* and a monophagous race infecting only *P. paniculata* and *Drummondii* are known. The importance of a knowledge of alternative hosts in averting infections is evident.

Mr D. E. Green gave an account of the history of the introduction and spread of *Antirrhinum* rust *Puccinia Antirrhini* in this country, and of the attempts to control it carried out at Wisley. The only effective treatment is the breeding of immune varieties. Resistance is regarded as a simple dominant factor, and breeding work has been largely directed to obtaining forms of good colour.

EXPERIMENTAL TAXONOMY

IV. POPULATION DIFFERENTIATION IN NORTH
AMERICAN AND EUROPEAN SEA PLANTAINS
ALLIED TO *PLANTAGO MARITIMA* L.

By J. W. GREGOR

Scottish Plant Breeding Station, Corstorphine, Edinburgh

(With 5 figures in the text)

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I. INTRODUCTION

THE present investigation is primarily concerned with the racial divergence within the diploid population of sea plantains allied to *Plantago maritima* L., and is based on the detailed study in an experimental garden of representative samples of small communities whose members are likely to breed more often amongst themselves than with members of other communities in the vicinity. In culture the separate samples have been found almost invariably to possess an individuality of their own; an individuality which is often only perceptible by the *majority* of individuals in a sample resembling each other more than they resemble the *majority* of individuals of another sample. The differences, however, cannot always be expressed in terms of variates possessed by one sample and not by another. Nevertheless, within the total population the unequal distribution of certain characters makes it possible to detect communities possessing attributes peculiar to themselves. But since the territories covered by such distinctive characters are seldom entirely coincident, the chances of identifying populations by an association of these attributes in relatively stable combination is not great.

The customary practice of according taxonomic recognition only to those populations which show a relatively high degree of association (fortuitous and otherwise) involving several "diagnostic" characters, would tend to obscure the geographical and ecological relationships of individual characters and character variations. By such treatment populations of considerable evolutionary consequence might even fail to find a place in the taxonomic scheme, e.g. an ecological race exhibiting a low degree of character association which extends through the territories

occupied by two or more populations of high character association, or populations which are separated by sterility barriers unaccompanied by appreciable morphological divergence. It is just such anomalies as these which tend to focus attention on the apparent artificiality of orthodox taxonomy. But as Gilmour (1937) has pointed out in a valuable article on the logic of classification, "it is usually stated in logic that a system of classification is the more natural the more propositions there are that can be made regarding the classes". Judged then by this standard, most biologists will agree that the present morphological classification of organisms is the most "natural" one yet devised. And, as the difference between a natural and an artificial classification is only one of degree, the incorporation of additional data into the traditional system could only result in raising its degree of naturalness. Nevertheless, until more is known of the specific and infra-specific categories of variation in the wild, a too precipitate amalgamation of experimental and traditional taxonomy would almost inevitably have repercussions detrimental to the present usefulness of the established morphological system. Therefore in the meantime it would seem more appropriate for experimental taxonomists to refrain from attempting to meet their requirements by any redefinition of the orthodox classificatory categories, but instead to use a complementary system of classification with a distinctive terminology, at least until the true value of new categories, and their possible place in a more comprehensive system, can be assessed. Whether or not an intensive experimental study of populations will of itself lead ultimately to a classification more natural (in the sense of Gilmour) than the existing one cannot be foreseen, but there is little doubt that an additional source of classified data relative to the constitution of species-segregates will have its value.

Table I. *Source of material*

Ref. no.	Locality	Habitat notes	Date collected	Collector
56	N. America, west coast Montaro lighthouse, California	Ocean bluff; granitic soil; competition	14. xi. 30	Prof. Leroy Abrams
57	Montaro lighthouse, California	Ocean bluff; granitic soil; no competition	14. xi. 30	do.
58	Montaro lighthouse, California	Road excavation; granitic soil; no competition	14. xi. 30	do.
85	Salado, California	Coastal dunes	23. x. 32	Dr J. Clausen
86	Sitka, Alaska	—	— 32	Dr Eric Hulten
59	N. America, east coast Charlottetown, Prince Edward Island	Swamp at seaside	22. ix. 31	Per Dr O. McConkey
60	Margertsville, Nova Scotia	Rocky cliffs, Bay of Fundy shore	22. ix. 31	Mr J. F. Hockey
61	Hortonville, Nova Scotia	Just about high-tideline, meadow dykeland	21. ix. 31	do.
73	Clondorme, Gaspé County, P.Q.	—	25. vii. 32	Mr J. Rausseau
81	Scituate, Mass.	Salt marsh	11. x. 32	Mr C. A. Weatherby
82	Momauguin, New Haven, Conn.	Salt marsh	14. ix. 32	J. W. G.
83	Point of Pines, Boston, Mass.	Salt marsh	12. ix. 32	do.
84	Gloucester, Mass.	Rocks by the sea	16. x. 32	Mr C. A. Weatherby
89	Bar Harbor, Me.	Maritime rocks	15. vii. 34	Dr G. G. Hahn

Table I (continued)

Ref. no.	Locality	Habitat notes	Date collected	Collector
52	Greenland Godhavn, W. Greenland	Gneiss cliffs (coastal)	25. ix. 30	Mr M. P. Porsild
66	N. Europe, Iceland Reykjavik	—	30. vii. 30	Per Mr C. Rasmussen
93	Isafjordur (N.W.)	Maritime rocks and gravel, above high-tide line	8. ix. 35	J. W. G.
94	Midfjordur (N.W.)	10 km. inland, stony soil, thinly populated	12. ix. 35	do.
95	Saurbaer (W.)	1 km. inland, stony hillside	13. ix. 35	do.
96	Reykjavik	Turf hummocks, from high-tide line to 100 yd. inland	14. ix. 35	do.
97	Hafnarfjordur (W.)	Rocky hill (coastal)	7. ix. 35	do.
98	Stadur (S.W.)	A rift in lava containing fresh water	20. ix. 35	do.
99	Reykjanes (S.W.)	Pasture on lava soil	20. ix. 35	do.
100	Reykjanes (S.W.)	Vicinity of hot spring "Geysir"	20. ix. 35	do.
102	Reykir (S.)	Neighbourhood of "Gryta" geysir	5. ix. 35	do.
103	Great Geysir	Neighbourhood of Great Geysir	5. ix. 35	do.
104	Hlidarendi (S.)	Gravelly river bed 22 km. inland	16. ix. 35	do.
105	Mulakot (S.)	Rocky hillside, 350 ft. above sea-level	16. ix. 35	do.
106	Skogafoss (S.)	Gravelly river bed	18. ix. 35	do.
107	Portland (S.)	Exposed cliff top	19. ix. 35	do.
108	Vik (S.)	Sandy rock ledges (coastal)	19. ix. 35	do.
109	Solheimajökull (S.)	Almost unpopulated moraines close to glacier	18. ix. 35	do.
51	N. Europe, Faroes Højvig, Thorshavn	Maritime rocks	25. viii. 30	Mr E. Knudsen
110	Thorshavn	Maritime rocks	2. ix. 35	J. W. G.
	N. Europe, Britain	Thirty-three localities (see Gregor, 1938)		
114	N. Europe, Norway Svolvaer, Lofoten Islands	Coastal mud at tide mark	3. vii. 38	J. W. G.
42	N. Europe, Sweden Landskrona	Sandy soil (coastal)	30. x. 29	Dr G. Nilsson-Leissner
43	Svälöf	85 m. above sea-level, 30 km. inland	7. x. 29	do.
53	Vitgrundet Island	Northernmost point of the Baltic Sea	16. vii. 30	do.
115	N. Europe, Finland Liinahamari (N.)	Salt marsh	28. vi. 38	J. W. G.
115a	Nurmensatti (N.)	Maritime rocks	27. vi. 38	do.
62	Alps, S. Germany Mittenwald-Krünn road, Bavaria	Banks of river Isar, 900 m. above sea-level	14. ix. 31	do.
63	Seefeld-Mosern path, Ostmark	Meadow, 1240 m. above sea-level	16. ix. 31	do.
65	Scharnitz, Ostmark	Dolomite, 960 m. above sea-level	11. ix. 31	Drs J. Clausen and H. Gams
111	Söllereck, Allgäu	Alp, 1400-1700 m. above sea-level	19. vii. 36	Dr M. J. F. Gregor
112	Söllereck, Allgäu	Alp, 1100 m. above sea-level	19. vii. 36	do.
113	Gaisberg, Allgäu	Alp, 1200-1382 m. above sea-level	— viii. 37	Frau von Franqué
113a	Aelple, Allgäu	—	— viii. 37	do.
116	Alps, Switzerland Interlaken	—	20. viii. 38	Per Dr R. Jenzer
117	Geneva	—	20. viii. 38	Per Prof. F. Chodat
118	Davos	—	20. viii. 38	Per Prof. A. Ernst
119	Fexthal	2400 m. above sea-level	— ix. 38	Dr J. M. Cowan
130	Alps, N. Italy Torino	Alpine pasture	28. xi. 38	Prof. V. Vezzani

Table II.
(See Table I, Gregor (1938), for

Region	Sample no.	Habit grade	Scape spread:height	Scape spread in.	Scape height in.	Scape length cm.	Scape thickness mm.	Spike length cm.	
N. America: Western	56	1'96	1'72±0'034	15'8±0'26	9'4±0'18	28'0±0'38	2'01±0'031	9'00±0'205	
	57	1'79	1'94±0'051	13'1±0'33	7'1±0'22	23'0±0'58	1'95±0'036	7'08±0'233	
	58	1'92	1'68±0'040	13'3±0'31	8'1±0'20	23'5±0'39	1'73±0'027	7'16±0'189	
	86	2'65	1'38±0'023	10'4±0'27	7'6±0'19	21'2±0'43	1'32±0'018	6'89±0'132	
Eastern	60	2'97	1'30±0'016	16'5±0'24	12'8±0'18	33'1±0'34	1'58±0'017	8'35±0'115	
	84	2'98	1'26±0'014	14'0±0'24	11'2±0'18	29'4±0'42	1'58±0'023	9'32±0'181	
	89	3'02	1'09±0'011	12'9±0'15	12'0±0'12	29'7±0'22	1'71±0'016	8'14±0'136	
	81	3'18	0'90±0'020	9'5±0'31	10'5±0'27	29'1±0'52	1'68±0'023	10'05±0'236	
82	3'90	0'54 —	6'4 —	11'9 —	31'5 —	—	9'27 —		
N. Europe: Iceland	66	2'29	1'38±0'019	12'3±0'19	9'0±0'15	24'1±0'36	1'29±0'018	6'80±0'151	
	93	1'99	1'62±0'048	13'5±0'27	8'8±0'23	27'2±0'53	1'63±0'023	7'35±0'179	
	99	1'95	1'73±0'104	11'4±0'38	7'2±0'35	23'2±0'77	1'67±0'036	7'51±0'254	
	100	1'23	2'51±0'078	8'3±0'24	3'6±0'14	14'0±0'45	1'21±0'023	4'18±0'169	
	104	2'39	1'44±0'026	13'6±0'25	9'6±0'19	27'9±0'45	1'64±0'023	7'71±0'167	
	108	2'13	1'56±0'047	13'5±0'46	8'9±0'38	26'5±0'94	1'72±0'033	6'64±0'245	
	Faroes	51	1'92	1'59±0'039	11'0±0'26	7'2±0'19	21'4±0'53	1'34±0'025	5'76±0'166
		110	1'94	1'77±0'065	12'6±0'48	7'5±0'34	23'1±0'80	1'49±0'029	6'10±0'252
Britain	(range)	1'21-3'33	0'81- 2'27	8'5-24'8	4'1-22'4	14'9-53'8	1'22- 2'70	3'82-13'96	
Sweden	42	3'07	0'90±0'015	20'0±0'40	22'4±0'26	51'9±0'60	2'01±0'022	9'46±0'196	
	43	3'43	0'91±0'014	17'3±0'29	19'2±0'22	48'0±0'58	1'83±0'016	11'54±0'180	
N. American	(range)	1'79-3'90	0'54- 1'94	6'4-16'5	7'1-12'8	21'2-33'1	1'32- 2'01	6'89-10'05	
N. European	(range)	1'23-3'43	0'81- 2'51	8'3-24'8	3'6-22'4	14'0-53'8	1'21- 2'70	3'82-13'96	
Alps: Allgäu	111	2'61	1'48±0'019	11'4±0'21	7'7±0'13	17'9±0'34	1'53±0'023	3'77±0'077	

Region	Sample no.	Habit grade	Bract length mm.	Bract breadth mm.	Bract index	Sepal length mm.	Sepal breadth mm.	Sepal index
N. America: Western	56	1'96	2'63 ± 0'034	1'60 ± 0'016	1'65 ± 0'019	2'42 ± 0'016	—	—
	57	1'79	2'48 ± 0'030	1'53 ± 0'021	2'41 ± 0'015	2'41 ± 0'015	—	—
	58	1'92	2'50 ± 0'025	1'61 ± 0'017	1'55 ± 0'018	2'41 ± 0'014	—	—
	86	2'65	2'23 ± 0'031	1'67 ± 0'017	1'33 ± 0'013	2'01 ± 0'016	1'39 ± 0'008	1'45 ± 0'012
Eastern	60	2'97	2'35 ± 0'019	1'55 ± 0'014	1'52 ± 0'014	2'27 ± 0'012	1'41 ± 0'012	1'61 ± 0'014
	84	2'98	2'71 ± 0'028	1'48 ± 0'013	1'84 ± 0'015	2'28 ± 0'025	1'36 ± 0'014	1'66 ± 0'018
	89	3'02	2'88 ± 0'024	1'56 ± 0'015	1'86 ± 0'015	2'18 ± 0'012	1'28 ± 0'008	1'71 ± 0'012
	81	3'18	2'60 ± 0'020	1'62 ± 0'019	1'61 ± 0'016	2'29 ± 0'018	1'53 ± 0'013	1'50 ± 0'016
	82	3'90	2'88 —	1'77 —	1'64 —	2'40 —	1'60 —	1'52 —
N. Europe: Iceland	66	2'29	1'85 ± 0'023	1'01 ± 0'011	1'85 ± 0'018	2'03 ± 0'015	0'98 ± 0'009	2'10 ± 0'022
	93	1'99	2'12 ± 0'026	1'19 ± 0'014	1'80 ± 0'020	2'11 ± 0'019	1'09 ± 0'011	1'95 ± 0'019
	99	1'95	2'09 ± 0'044	1'22 ± 0'022	1'71 ± 0'027	2'00 ± 0'028	1'13 ± 0'016	1'78 ± 0'032
	100	1'23	1'88 ± 0'024	1'12 ± 0'013	1'69 ± 0'018	1'00 ± 0'011	1'00 ± 0'011	2'00 ± 0'019
	104	2'39	2'27 ± 0'024	1'22 ± 0'013	1'86 ± 0'019	2'12 ± 0'014	1'06 ± 0'010	2'02 ± 0'018
	108	2'13	2'15 ± 0'044	1'24 ± 0'017	1'73 ± 0'028	2'21 ± 0'024	1'10 ± 0'017	2'03 ± 0'035
	Faroes	51	1'92	2'39 ± 0'040	1'38 ± 0'020	1'75 ± 0'024	2'36 ± 0'023	—
110		1'94	2'03 ± 0'043	1'15 ± 0'018	1'78 ± 0'032	2'19 ± 0'033	1'07 ± 0'015	2'06 ± 0'033
Britain	(range)	1'21-3'33	1'99- 3'30	1'11- 1'69	1'62- 2'25	2'17- 2'81	1'02- 1'35	1'94- 2'34
Sweden	42	3'07	3'38 ± 0'032	1'54 ± 0'018	2'21 ± 0'026	—	—	—
	43	3'43	2'69 ± 0'033	1'15 ± 0'013	2'35 ± 0'023	2'32 ± 0'019	0'98 ± 0'011	2'38 ± 0'026
N. American	(range)	1'79-3'90	2'23- 2'88	1'48- 1'77	1'33- 1'86	2'01- 2'42	1'28- 1'53	1'45- 1'71
N. European	(range)	1'23-3'43	1'85- 3'38	1'01- 1'69	1'62- 2'35	1'98- 2'81	0'98- 1'35	1'78- 2'38
Alps: Allgäu	111	2'61	2'47 ± 0'027	1'31 ± 0'014	1'90 ± 0'019	1'98 ± 0'017	1'24 ± 0'012	1'59 ± 0'013

Mean values

means of samples from Britain.)

Scape volume litres	Leaf length cm.	Leaf breadth mm.	Leaf index	Leaf thickness mm.	Leaf spread in.	Leaf height in.	Leaf spread:height	Spike density
1.93 ± 0.086	14.1 ± 0.32	11.47 ± 0.235	12.6 ± 0.34	1.33 ± 0.029	3.38 ± 0.148	1.23 —	2.82 ± 0.097	12.2 ± 0.19
1.08 ± 0.084	12.0 ± 0.38	11.09 ± 0.284	11.2 ± 0.41	1.31 ± 0.029	3.00 ± 0.135	1.09 —	2.77 ± 0.115	14.7 ± 0.30
1.19 ± 0.079	14.3 ± 0.39	12.24 ± 0.273	12.0 ± 0.37	1.16 ± 0.021	2.94 ± 0.163	1.14 —	2.65 ± 0.122	12.8 ± 0.24
0.73 ± 0.053	20.6 ± 0.42	5.90 ± 0.115	35.4 ± 0.64	0.85 ± 0.014	6.32 ± 0.194	1.84 ± 0.063	3.50 ± 0.070	12.1 ± 0.17
2.88 ± 0.102	26.1 ± 0.32	8.12 ± 0.154	32.9 ± 0.59	0.89 ± 0.014	11.28 ± 0.197	3.72 ± 0.065	3.10 ± 0.064	13.2 ± 0.14
1.81 ± 0.080	22.2 ± 0.35	7.37 ± 0.198	31.5 ± 0.62	0.98 ± 0.017	—	—	—	10.5 ± 0.15
1.60 ± 0.048	22.4 ± 0.26	5.56 ± 0.111	41.5 ± 0.86	1.14 ± 0.017	8.54 ± 0.189	3.71 ± 0.071	2.33 ± 0.043	12.1 ± 0.15
0.85 ± 0.071	23.0 ± 0.39	7.57 ± 0.197	31.3 ± 0.73	0.95 ± 0.016	—	—	—	8.5 ± 0.20
0.43 —	25.4 —	8.05 —	31.4 —	—	—	—	—	9.2 —
1.12 ± 0.047	15.4 ± 0.27	3.53 ± 0.093	45.1 ± 1.17	0.78 ± 0.010	6.99 ± 0.195	1.78 ± 0.047	4.01 ± 0.118	15.2 ± 0.21
1.31 ± 0.070	20.0 ± 0.40	4.73 ± 0.135	43.8 ± 1.10	0.93 ± 0.015	7.18 ± 0.239	2.34 ± 0.091	3.25 ± 0.094	14.8 ± 0.21
0.78 ± 0.078	14.6 ± 0.59	5.88 ± 0.268	26.1 ± 1.14	0.94 ± 0.018	5.51 ± 0.257	1.53 ± 0.092	3.85 ± 0.161	14.1 ± 0.30
0.25 ± 0.024	8.3 ± 0.32	3.00 ± 0.113	28.7 ± 0.79	0.89 ± 0.014	3.13 ± 0.114	0.82 ± 0.032	3.96 ± 0.115	16.7 ± 0.27
1.48 ± 0.074	21.0 ± 0.34	4.50 ± 0.133	49.3 ± 1.36	1.02 ± 0.014	8.85 ± 0.219	2.53 ± 0.075	3.64 ± 0.087	14.7 ± 0.17
1.42 ± 0.149	19.5 ± 0.77	6.23 ± 0.313	33.2 ± 1.42	0.96 ± 0.024	7.84 ± 0.421	2.35 ± 0.121	3.45 ± 0.126	15.2 ± 0.32
0.80 ± 0.056	13.9 ± 0.38	4.80 ± 0.144	29.9 ± 0.74	1.05 ± 0.013	4.92 ± 0.169	1.54 —	3.42 ± 0.108	14.3 ± 0.30
1.12 ± 0.116	15.6 ± 0.67	5.02 ± 0.240	32.8 ± 1.49	1.08 ± 0.020	6.77 ± 0.402	2.22 ± 0.140	3.29 ± 0.101	14.5 ± 0.27
0.29-11.18	11.5-36.1	3.87-12.20	27.0-52.2	0.77-1.33	3.48-19.51	1.04-7.08	2.48-4.47	11.1-15.0
7.53 ± 0.309	36.0 ± 0.68	10.83 ± 0.154	34.1 ± 0.68	1.24 ± 0.021	18.21 ± 0.271	7.41 ± 0.151	2.52 ± 0.043	11.3 ± 0.17
4.68 ± 0.183	34.2 ± 0.59	6.73 ± 0.125	51.7 ± 1.05	0.76 ± 0.011	13.82 ± 0.265	5.82 ± 0.145	2.45 ± 0.047	15.3 ± 0.21
0.43-2.88	12.0-26.1	5.56-12.24	11.2-41.5	0.85-1.33	2.94-11.28	1.09-3.72	2.33-3.50	8.5-14.7
0.25-11.18	8.3-36.1	3.00-12.20	26.1-52.2	0.76-1.33	3.13-19.51	0.82-7.41	2.45-4.47	11.1-16.7
0.83 ± 0.040	10.0 ± 0.22	7.78 ± 0.149	20.6 ± 0.45	0.73 ± 0.010	10.00 ± 0.185	3.47 ± 0.063	2.88 ± 0.032	17.6 ± 0.24

Seed length mm.	Seed breadth mm.	Seed index	Anther length mm.	Anther tip length mm.	Bract length:sepal length	Scape length:spike length	Scape length:leaf length	Flowering grade
1.67 ± 0.015	0.91 ± 0.0076	1.85 ± 0.013	1.88 ± 0.018	0.257 ± 0.0070	1.08 ± 0.0125	3.18 ± 0.045	2.01 ± 0.040	2.35 ± 0.127
1.74 ± 0.014	0.93 ± 0.0076	1.88 ± 0.014	1.88 ± 0.020	0.234 ± 0.0074	1.03 ± 0.0117	3.36 ± 0.066	2.01 ± 0.050	2.54 ± 0.132
1.72 ± 0.015	0.94 ± 0.0077	1.84 ± 0.011	1.81 ± 0.021	0.214 ± 0.0057	1.04 ± 0.0094	3.35 ± 0.053	1.79 ± 0.033	3.46 ± 0.210
2.24 ± 0.022	1.08 ± 0.0092	2.09 ± 0.017	1.74 ± 0.013	0.276 ± 0.0042	1.10 ± 0.0121	3.10 ± 0.041	1.04 ± 0.019	4.88 ± 0.149
1.94 ± 0.010	0.96 ± 0.0066	2.02 ± 0.014	1.79 ± 0.010	0.325 ± 0.0048	1.04 ± 0.0083	4.01 ± 0.045	1.28 ± 0.013	4.13 ± 0.119
1.79 ± 0.018	0.89 ± 0.0077	2.03 ± 0.018	1.52 ± 0.012	0.279 ± 0.0037	1.20 ± 0.0098	3.20 ± 0.033	1.33 ± 0.014	2.20 ± 0.083
1.70 ± 0.014	0.86 ± 0.0076	2.00 ± 0.017	1.57 ± 0.013	0.343 ± 0.0046	1.33 ± 0.0098	3.74 ± 0.061	1.36 ± 0.017	3.86 —
2.14 ± 0.024	0.92 ± 0.0127	2.33 ± 0.017	1.88 ± 0.015	0.281 ± 0.0048	1.14 ± 0.0136	2.92 ± 0.048	1.27 ± 0.017	1.00 —
2.20 —	0.90 —	2.46 —	—	—	1.20 —	3.31 —	1.23 —	1.23 —
2.32 ± 0.016	0.97 ± 0.0061	2.41 ± 0.017	1.78 ± 0.016	0.208 ± 0.0050	0.92 ± 0.010	3.65 ± 0.059	1.59 ± 0.021	6.18 ± 0.199
2.26 ± 0.018	0.99 ± 0.0076	2.28 ± 0.017	2.03 ± 0.018	0.254 ± 0.0071	1.00 ± 0.011	3.82 ± 0.073	1.39 ± 0.026	2.69 ± 0.191
2.20 ± 0.027	0.97 ± 0.0115	2.28 ± 0.023	1.89 ± 0.029	0.246 ± 0.0069	1.05 ± 0.018	3.13 ± 0.078	1.63 ± 0.045	2.90 ± 0.300
2.06 ± 0.019	0.93 ± 0.0078	2.23 ± 0.016	1.85 ± 0.021	0.223 ± 0.0051	0.95 ± 0.010	3.57 ± 0.087	1.75 ± 0.035	4.85 ± 0.201
2.23 ± 0.015	0.97 ± 0.0071	2.32 ± 0.015	1.96 ± 0.018	0.244 ± 0.0051	1.07 ± 0.012	3.70 ± 0.058	1.34 ± 0.019	3.83 ± 0.146
2.28 ± 0.024	1.02 ± 0.0114	2.24 ± 0.025	1.95 ± 0.029	0.244 ± 0.0107	0.98 ± 0.021	4.12 ± 0.148	1.37 ± 0.035	4.46 ± 0.299
2.34 ± 0.014	1.03 ± 0.0062	2.27 ± 0.014	2.10 ± 0.032	0.240 ± 0.0065	1.01 ± 0.016	3.66 ± 0.058	1.59 ± 0.037	5.09 ± 0.177
2.19 ± 0.023	0.98 ± 0.0104	2.24 ± 0.023	1.88 ± 0.022	0.266 ± 0.0064	0.94 ± 0.016	3.85 ± 0.076	1.52 ± 0.042	5.56 ± 0.267
2.03-3.01	0.92-1.13	2.22-2.79	1.80-2.58	0.234-3.35	0.92-1.22	3.20-4.81	1.18-1.82	1.01-7.64
2.52 ± 0.016	1.03 ± 0.0055	2.46 ± 0.013	2.62 ± 0.027	0.272 ± 0.0093	—	5.66 ± 0.107	1.54 ± 0.022	1.52 ± 0.077
2.19 ± 0.017	0.94 ± 0.0071	2.33 ± 0.011	1.90 ± 0.017	0.288 ± 0.0055	1.17 ± 0.013	4.21 ± 0.063	1.43 ± 0.021	1.09 ± 0.037
1.67-2.24	0.86-1.08	1.84-2.46	1.52-1.88	0.214-0.343	1.03-1.33	2.92-4.01	1.04-2.01	1.00-4.88
2.03-3.01	0.92-1.13	2.22-2.79	1.78-2.62	0.208-0.335	0.92-1.22	3.13-4.81	1.18-1.82	1.01-7.64
1.75 ± 0.015	0.82 ± 0.0073	2.14 ± 0.014	1.99 ± 0.015	0.235 ± 0.0043	1.26 ± 0.013	4.86 ± 0.087	1.83 ± 0.032	9.94 ± 0.075

Table III. *Variate ranges (Summary)*
(For full character headings see Table II)

	HbG.	ScS.:H.	ScS.	ScH.	ScL.	ScTh.	SpL.	ScV.	LfL.	LfB.	LfFx.	LfTh.	LfS.	LfH.	Lfs.:H.	SpD.
Region:																
N. America	1-5	0.4-3.3	4-22	3-17	11-42	1.0-2.8	4-16	0.1-5.3	6-32	3-19	6-65	0.5-1.8	1-16	1.0-5.5	1.0-7.0	6-21
N. Europe	1-5	0.5-7.1	3-31	1-29	5-69	0.7-3.5	1-21	0.1-20.4	3-56	1-22	13-100	0.5-2.0	1-28	0.5-13.0	1.0-9.0	7-23
Alps	2-3	1.0-1.9	6-17	5-11	9-26	1.0-2.1	2-6	0.2-2.3	5-16	4-12	8-28	0.5-1.0	5-14	2.0-5.5	1.9-3.7	12-25
Subregion:																
W. America	1-3	1.0-3.3	6-21	3-13	11-38	1.0-2.8	4-14	0.1-3.8	6-29	3-19	6-50	0.5-1.8	1-11	1.0-2.5	1.0-7.0	8-21
E. America	1-5	0.8-1.8	4-22	6-17	20-42	1.0-2.2	4-16	0.1-5.3	12-32	3-12	19-65	0.6-1.6	4-16	2.0-3.5	1.0-3.3	6-17
Iceland	1-4	0.8-1.8	3-19	2-15	6-39	0.7-2.3	1-12	0.1-9.5	5-32	1-12	13-95	0.6-1.1	1-15	0.5-2.5	2.0-3.3	10-23
Faroes	1-4	1.0-3.5	3-19	2-15	9-40	0.9-2.1	2-12	0.1-3.8	5-32	2-10	14-60	0.7-1.4	1-15	1.0-3.5	1.0-3.3	10-23
Britain	1-5	0.8-7.4	4-31	1-27	5-69	0.7-3.5	1-21	0.1-20.4	3-51	1-22	13-100	0.5-2.0	2-28	1.0-13.0	1.0-9.0	10-20
S. Sweden	1-5	0.6-1.4	9-28	13-24	35-68	1.4-2.6	6-16	1.0-14.2	17-56	4-15	19-86	0.6-1.8	7-23	2.5-10.5	1.0-9.0	7-22

	BrL.	BrB.	BrFx.	Sep.L.	Sep.B.	Sep.Ix.	SdL.	SdB.	SdIx.	AL.	ATL.	BrL.:SepL.	ScL.:SpL.	ScL.:LfL.
Region:														
N. America	1.71-3.77	0.99-2.06	1.1-2.3	1.63-2.79	0.94-1.84	1.2-2.3	1.33-2.79	0.678-1.29	1.6-2.8	1.29-2.27	0.120-0.472	0.8-1.5	2.3-5.8	0.7-3.5
N. Europe	1.81-4.28	0.77-2.23	1.1-3.2	1.54-3.30	0.73-1.67	1.3-3.2	1.63-3.51	0.772-1.40	1.8-3.5	1.33-3.09	0.080-0.472	0.7-1.5	1.8-8.8	0.7-3.2
Alps	1.80-3.39	0.99-1.63	1.5-2.6	1.50-2.49	0.99-1.54	1.3-1.9	1.41-2.14	0.686-0.99	1.8-2.4	1.63-2.36	0.120-0.343	1.0-1.7	3.2-7.7	1.1-2.9
Subregion:														
W. America	1.71-3.39	1.20-2.06	1.1-2.0	1.63-2.79	1.20-1.54	1.2-1.8	1.33-2.66	0.772-1.29	1.6-2.5	1.50-2.27	0.129-0.386	0.8-1.5	2.3-5.0	0.7-3.5
E. America	1.97-3.77	0.99-1.97	1.1-2.3	1.76-3.26	0.94-1.84	1.3-2.3	1.37-2.79	0.673-1.24	1.6-2.8	1.29-2.14	0.23-0.472	0.9-1.5	2.3-5.8	0.7-3.5
Iceland	1.41-2.83	0.77-1.50	1.3-2.5	1.54-2.57	0.77-1.37	1.3-2.8	1.63-2.79	0.772-1.24	1.7-2.9	1.33-2.36	0.086-0.472	0.9-1.4	2.3-5.8	0.9-1.8
Faroes	1.50-2.70	0.90-1.50	1.3-2.5	1.70-2.70	0.90-1.29	1.6-2.9	1.80-2.91	0.814-1.24	1.7-2.9	1.50-2.91	0.171-0.386	0.8-1.2	2.8-3.3	0.9-2.8
Britain	1.50-4.10	0.81-2.14	1.3-3.2	1.67-3.39	0.90-1.67	1.5-3.2	1.63-3.51	0.772-1.46	1.8-3.5	1.46-3.99	0.086-0.472	0.7-1.5	1.8-8.8	0.7-3.2
S. Sweden	1.97-4.28	0.90-2.23	1.6-2.9	1.89-2.79	0.73-1.24	1.8-3.0	1.80-3.21	0.814-1.20	2.0-2.9	1.54-3.99	0.086-0.429	0.9-1.5	3.1-8.8	1.0-2.3

The present investigation has involved the examination of living material in the experimental garden at Corstorphine. The collection of seed samples from various habitats, the subsequent cultivation of populations, and the sampling of individual plants were carried out in accordance with the methods found by preliminary experimentation to be the most reliable. These methods have already been described (Gregor *et al.* 1936, pp. 327-8). Briefly the technique was as follows: Ripe spikes were systematically gathered from comparatively well-defined but not necessarily spatially isolated habitats; seeds were sown in weed-free soil and later 102 seedlings of each sample were transplanted into the experimental garden where they were examined in their second year. Every plant received a number, and the data relating to each were recorded. A list of the samples is given in Table I.

II. THE ANALYSIS OF MEASURABLE CHARACTERS

(a) *The comparison of average character values*

In Table II the mean values are given for the various characters of the samples examined, while in Table III the variate ranges are given for the same characters. In both these tables the samples have been arranged according to their geographical source by regions (A) North American, (B) North European and (C) Alpine (from the Alps). (A) has been again subdivided into subregions (a) Western North America, and (b) Eastern North America, and (B) into (a) Iceland, (b) Faroes, (c) Britain and (d) South Sweden. Several samples represented by relatively few individuals have been omitted from Tables II and III.

When making comparisons between any two of these regions or subregions an average character value cannot be usefully employed for all characters. For example, it will be found that three out of the four samples from Western America are derived from somewhat similar habitats in California, and, since growth habit and the size of organs have been shown to be influenced by ecological conditions (Gregor, 1938), the average of the mean values for these characters is probably biased by the predominance of Californian samples. There are, however, other characters, e.g. anther length, which, being less influenced by ecological conditions, vary locally in a more or less random fashion. These characters have therefore been chosen as the most reliable indicators of average regional differentiation. Since the subregional means differ considerably and since these means are based on unequal numbers of samples, the regional averages are liable to be biased in favour of the subregion supplying the greatest number of samples. For example, in North Europe the British samples outnumber those from any other country, and in consequence the North European average reflects this excess. By using the method of analysis of variance, analysing the variations of means into portions attributable to regions and subregions, it is possible to overcome this bias to some extent, and significant differences between regions and between subregions have been proved to exist. The 1% level of the distribution of Z (Fisher, 1932, Table VI) has been taken as the criterion of significance.

It will be seen from Table IV that the American samples differ from the North European for the characters bract, sepal and seed indices, anther length and scape

length:spike length. Therefore, although, as will be seen from Table II, the regional mean ranges are continuous this analysis demonstrates that on the average the North American samples have bracts, sepals and seeds relatively broader in proportion to length than those of North Europe.

Table IV. *The comparison of regional average values*

Character	Regions		Subregions					
	N. America	N. Europe	W. America	E. America	Iceland	Faroes	Britain	S. Sweden
Seed index	2.06 *	2.44	1.92	2.17	2.29	2.26	2.49	2.40
Bract index	1.61 *	1.93	1.54	1.66	1.77	1.77	1.97	2.09
Sepal index	1.58 *	2.08	1.45	1.60 *	1.98	2.06	2.12	2.14
Bract length: sepal length	1.13	1.05	1.06	1.17 *	1.00	0.98	1.07	1.08
Anther length	1.76 *	2.10	1.83	1.69	1.91	1.99	2.15	2.09
Anther tip length	0.28	0.27	0.25	0.31 *	0.24	0.25	0.27	0.29
Scape length: spike length	3.33 *	3.92	3.25	3.39	3.67	3.76	3.91 *	4.99
Scape length: leaf length	1.48	1.49	1.71	1.30	1.51	1.55	1.49	1.49

* Indicates a significant break between adjacent values.

The analysis of variance has also shown that significant differences are to be found between subregions for seven out of the eight characters examined, scape length:leaf length being the exception. In Table IV the subregions have been arranged in geographical sequence, and the significance of the differences between adjacent average values has been determined from Fisher's table of *t* (Fisher, 1932, Table IV). It will be noted that, while lacking significant breaks in the series, the North European subregions exhibit a tendency for the bracts and sepals to become progressively narrower in relation to length from Iceland towards South Sweden. Of the five characters for which significant differences have been established between North America and North Europe only one, sepal index, shows a break in the range of mean values between Atlantic America and Iceland, demonstrating that the transition from the one region to the other is more or less gradual.

(b) *The comparison of the range of character mean values*

For reasons which have been already stated regarding the limited usefulness of comparisons between the regional and subregional average values, the ranges of the mean values have been taken as the general basis of comparison. Breaks in continuity of these ranges have been regarded as significant when the difference between the highest mean of one region or subregion and the lowest of another exceeds three times its error; e.g. for the character scape height, a break occurs between the western and eastern North American subregions, the highest value for West America is 9.4 ± 0.18 in. and the lowest for East America is 10.5 ± 0.27 in., a difference of 1.1 ± 0.32 in.

American and North European samples compared. The character sepal index provides the only break between the North American and North European ranges

of sample means, a break which is not significant. However, as Iceland is the part of Europe nearest the American Continent it is of interest to compare the range of mean values of samples for this subregion with that for Atlantic North America. Between these two subregions significant gaps occur for the characters bract and sepal breadth, anther tip length and scape spread:height. Nevertheless, the geographic continuity of all these characters is preserved by the overlapping of the variate ranges, as may be seen by reference to Table III.

American samples compared. An examination of the mean ranges shows that the mean values of samples from the east and west coasts overlap, with the exception of those of scape length and height; scape spread:height; habit grade; leaf length, spread and height; sepal index and anther tip length. Of these breaks, however, only those for scape height, leaf spread and height and possibly habit grade (a character for which there is no reliable error available) are statistically significant. That is, the subregional discontinuity is confined to measurements of growth-habit characters. But when reference is made to Table III it will be seen that the discontinuity does not extend to the variate ranges, every character exhibiting overlapping to some extent.

A glance at this table will show how evident it is that even when certain sample mean values deviate distinctly from others in respect of the same character, an individual plant might equally well belong to any one of a number of different samples. For example, the most decumbent specimens in California are no more decumbent than specimens found on the Atlantic coast, and the most upright Californian specimens have their equivalents in all the Atlantic samples. Moreover, every one of the eastern American samples contains specimens having broader leaves than the narrowest leaf forms of western America, although, on the average, the Californian samples have significantly broader leaves than any samples from the east coast.

The mean and variate range values having thus failed to provide a separation of the American sea plantains into a Pacific and an Atlantic type, it is now necessary to examine the American population as a whole and endeavour to discover whether a more localized differentiation has taken place in this area. Of all the American samples the three from California (samples 56, 57 and 58) were the most striking to the casual observer on account of their decumbent scapes and broad leaves. The mean values bear out the truth of this observation and demonstrate that, in addition to their differing from all other American samples for the characters scape spread:height and leaf breadth, they also differ significantly in respect of leaf length (short), leaf index (broad in relation to length), scape length:leaf length (leaves considerably shorter than scapes) and seed index (seeds relatively broad in relation to length).

The next most obvious difference between samples was that distinguishing samples 81 and 82 from the remainder of the American populations, these two samples being characterized by their upright growth habit and the relative laxity of their spikes. Again these differences are confirmed by reference to Table II where it will be found that the mean values for scape spread:height and spike density differ

significantly from other American samples. With the exception of seed index (seeds relatively long in comparison to breadth) no other measurable difference of importance occurs.

Since a more dense-spiked race also occurs on the New England coast an examination is advisable of the character mean values of samples of both spike types from this restricted geographical area. If samples 81 and 82 be regarded as representative of the lax-spiked type and 84 and 89 of the more dense type, then a simultaneous comparison can be made between samples from salt-marsh and maritime rock habitats. The result is that the salt-marsh samples (81 and 82), in addition to having spikes more lax and scapes more erect than the maritime rock samples (84 and 89), differ significantly from the latter in that (1) their bracts and sepals, as shown by the respective indices, are broader in relation to length; (2) their seeds have greater absolute length, and are proportionately narrower; and (3) their anthers are larger. Davey & Lang (1939) give some evidence indicative of a tendency for the bract dimensions, in particular bract breadth, to be negatively correlated with the density of the spike. Lax-spiked individuals with relatively broad bracts are, however, not peculiar to the salt-marsh habitats, as may be seen from Table III. But the fact that plants with lax spikes and plants with relatively dense spikes predominate respectively in salt-marsh and maritime rock habitats suggests that, in respect of this character, population differentiation has occurred in response to ecological conditions. The supremacy of lax-spiked plants on other salt marshes of the Atlantic coast is also evidenced by the data obtained from samples 59, 83 and 61. Although these samples only contained twenty-five plants each, and therefore have not been included in the table of means, their spike density values 7.23, 7.50 and 8.33 respectively, are of interest in that they are all low in comparison with the samples 84, 89 and 60 from rock habitats.

A cross between a compact-spiked plant in sample 56 from California and a lax-spiked plant from sample 81 gave an F_1 generation of sixty-four plants having a mean spike density value of 6.94 ± 0.200 , a figure almost identical with that of 7.0 recorded for the male parent. A population of sixty-four plants was also grown from seed obtained by self-pollinating the Californian female parent; this population had a spike density value of 12.70 ± 0.231 . Thus in eastern America the presence of a dominant gene is apparently responsible for this lax-spike character, a character which has not been expressed to the same degree in any other region from which samples have been examined.

North European samples compared. No significant breaks in the mean value ranges occur between the subregions, provided the countries are taken in the geographical sequence: Iceland, Faroes, Britain and South Sweden. Such continuity makes the delimitation of sharply defined regional races impossible. Nevertheless, if the range of the South Swedish samples is compared with the ranges of the samples from Iceland and the Faroes, discontinuities are to be found in many characters. These characters are those descriptive of size and growth habit, with the exception of the bract and sepal indices and bract length: sepal length. These data suggest that maximum plant size is greater in Britain and South Sweden than it is in Iceland and

the Faroes, and also that the bract and sepal indices and the ratio bract length:sepal length reach their highest expression towards the south of this region. Although Britain possesses populations which exceed those of Iceland in erectness and plant size these two countries are alike in having populations of dwarf, decumbent plants, e.g. the Icelandic sample 100 and the British sample 47. Moreover, while there are British populations (sample 21) with bracts of narrower proportions than any recorded from Iceland, yet at the other extreme there is no significant difference between these subregions (samples 36 and 100). With the exception of that of scape height the variate range for North Europe is continuous.

The single Alpine sample 111 is separated significantly from all North European mean values in respect of leaf index (low), seed measurements (low seed index) and sepal index (low). However, with two minor exceptions, the variate ranges for these three characters fall completely within the North European range.

III. THE ANALYSIS OF NON-MEASURABLE CHARACTERS

(1) *Leaf pubescence*. The leaves were examined under a 12× lens, and the distribution and density of hairs was classified into nine arbitrary grades: (1) glabrous, (2) very few scattered hairs on margins, (3) few scattered hairs on margins, (4) a considerable number of hairs on margins, (5) hairs on margins and very few scattered hairs on lower surface, (6) hairs on margins and scattered hairs on lower surface, (7) hairs on margins and scattered hairs on upper surface, (8) hairs on margins and scattered hairs on both surfaces, and (9) thinly pubescent.

Table V. *Leaf pubescence. Percentage plants in each grade*

Subregions	Grades									Percentage plants in grades 4, 6 and 9
	1	2	3	4	5	6	7	8	9	
West America	0.8	22.8	30.3	21.4	12.4	9.9	1.1	1.0	0.4	31.7
East America	0.5	8.5	7.8	2.0	9.2	5.0	2.2	23.1	41.7	48.7
Iceland	0.0	0.2	1.6	3.4	8.9	42.4	0.0	6.3	37.2	83.0
Faroes	0.0	1.1	4.7	10.6	38.2	33.4	1.0	7.1	4.2	48.2
Britain	2.8	51.3	12.2	6.2	10.2	14.0	0.8	2.8	2.3	22.5
South Sweden	1.0	63.3	10.0	3.7	16.8	3.2	1.1	1.1	0.0	6.9
Alps (Allgäu)	0.6	17.3	7.0	3.3	38.4	23.0	0.0	10.0	0.6	29.9

Table V gives the percentage number of plants occurring in the different grades, summarized for the subregions. All nine grades are represented in both America and Europe. West America, Britain and Sweden have high percentages of plants with hairs confined to the leaf margins, whereas in the majority of plants from East America, Iceland and the Faroes, the hairs are more generally distributed over the leaf surface. By totalling the percentages for grades of maximum pubescence for the margins, lower surfaces, and both surfaces, viz. 4, 6 and 9, an idea can be obtained of the relative differences in hair density between subregions. By this standard of comparison the Icelandic samples are the most densely hairy, and the American samples fall within the European range. A further indication of hair density may be obtained from Table VI where the number of hairs per 4.29 mm. of the leaf margin and the lengths of the longest hairs in this distance are recorded for

six samples from East America, the same number from North Europe, and three samples from the Alps.

Table VI. *Density and length of leaf margin hairs*

Region	No. of hairs per 4.29 mm.		Hair length in mm.	
	Range of means	Variate range	Range of means	Variate range
East America	2.29-10.68	0-23	0.065-0.22	0.043-0.51
North Europe	1.35-13.78	0-24	0.095-0.47	0.043-1.46
Alps (Allgäu)	4.47-13.44	0-33	0.091-0.11	0.043-0.21

(2) *Leaf tooth grade*. The margins of sea plantain leaves may be entire or toothed, the length of the teeth varying greatly even in a single plant. To determine sample differences for this character the longest tooth per plant, as judged by eye, was measured in mm. and the measurements recorded in grades as follows: teeth of 1 mm. or less were denoted by the figure 1, teeth of 1.5 mm. by 2, teeth of 2.0 mm. by 3, and so on. The results are given in Table VII.

Table VII. *Leaf tooth grade*

Region	Variate range	Mean	Range of means
West America	1- 5	1.01	1.00-1.04
East America	1- 7	1.08	1.00-1.25
Iceland	1- 4	1.09	1.03-1.26
Faroës	1- 9	1.68	1.23-2.73
Britain	1-13	1.64	1.02-2.81
South Sweden	1- 9	1.98	1.23-2.73
Alps (Allgäu)	1- 9	1.54	1.50-1.57

(3) *Leaf spot*. The presence of anthocyanin leaf spots is a feature of many samples. The spots are usually small and of a deep wine-red colour and can best be observed in early spring and late autumn. The lack of spots is a recessive condition, and Dr Black of this Institute, who has grown an F_2 population of green \times spotted plants under conditions favourable to the expression of this character, finds that the ratio spotted:green is as 3:1 (260:79). The frequency of spotted-leaved plants has varied widely from sample to sample even locally. The results of the observations are summarized in Table VIII and it will be noticed that while the character is present in East America, Greenland and in all the subregions of North Europe, it has not been recorded from West America or from the Alps.

Table VIII. *Percentage frequency of spotted-leaved plants*

Subregion	Means	Ranges
West America	—	—
East America	73	3-100
Greenland	76*	—
Arctic Europe	78†	55-100
Iceland	72	40- 98
Sweden	64	52- 76
Faroës	8	4- 12
Britain	21	0- 63
Alps	—	—

* One sample.

† Unreliable, counted in the wild.

(4) *Ciliation of floral parts.* To record the observations arbitrary classes were devised after a preliminary survey had established the approximate limits of ciliation. The samples chosen for special comparison are 81 and 84, sent to the writer by Mr Weatherby of the Gray Herbarium under the respective names of *P. oliganthos* R. & S., and *P. juncoides* Lam. var. *decipiens* (Barnéoud) Fern., and sample 89, collected by Dr Hahn. These three samples come from the Atlantic American region where, as mentioned previously, differentiation has apparently taken place of a lax-spiked race of salt marshes (sample 81) and a dense-spiked maritime rock race

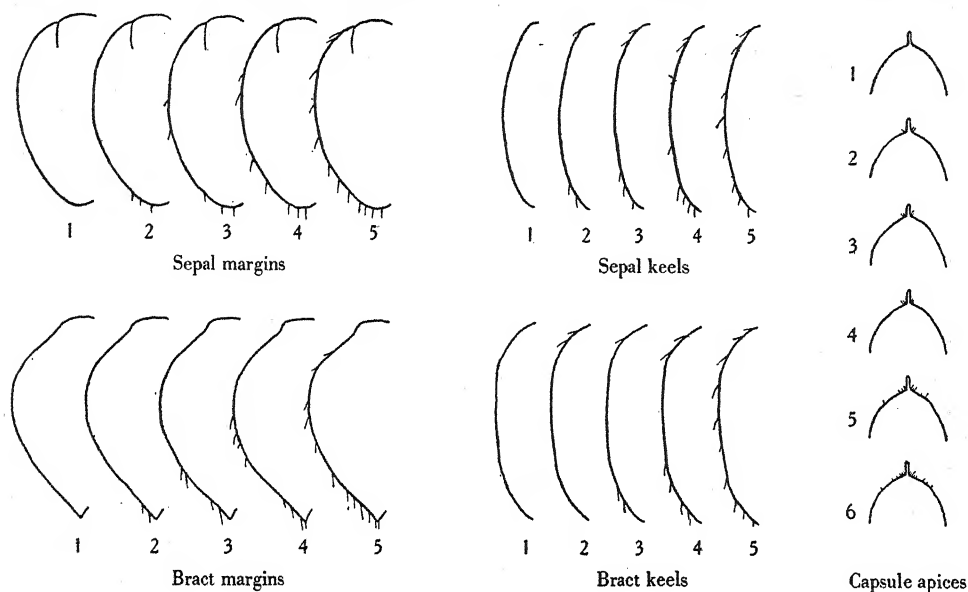


Fig. 1. Ciliation grades.

(samples 84 and 89). The two samples from maritime rocks differ considerably, which suggests that the expression of ciliation is independent of the rock environment. The sepal keels of sample 81 are glabrous in contrast to the varying degree of ciliation observed for samples 84 and 89, and there is evidence that the bract keels and sepal margins of 81 are also decidedly less ciliate. The position held by sample 81 with regard to the ciliation of the bract margins and capsule apices is not unique (Table IX).

Table IX. *Ciliation of floral parts in per cent*[illegible]

An F_1 population (a plant of sample 56 (California) \times a plant of sample 81) gave, for the five characters, percentages which fell midway between those recorded for sample 81 and those obtained for a population raised from selfed seed off the female parent.

No division can be drawn between the American and North European samples. The averages of the grade percentages for America and North Europe indicate that, for sepal margin and keel and capsule apex, the North European population is the more ciliated, while for bract margin and keel the values are very similar. However, these average percentages have little or no regional significance because of the great intraregional differences observed, e.g. the greatest and the least degree of ciliation of the bract margins was recorded from North European samples.

(5) *Number of seeds per capsule.* Dowling (1936) dissected forty young capsules of *P. maritima* L. (British material) and of these thirty-nine showed three ovules. Her conclusion is that "in *P. maritima* there are usually only three ovules (p. 335). . . usually two of these ovules abort, giving a mature capsule with one seed" (p. 336).

In the samples from Europe two viable seeds per capsule is the typical maximum number, but in almost every sample examined occasional plants were found with a few capsules containing three or even four developed seeds. The North American and Greenland samples, on the other hand, were found to have capsules with four developed seeds as the typical number, although most plants possessed a varying number of three-, two-, and less frequently one-seeded capsules. The number of seeds per capsule apparently affects to some extent the proportions of bracts and sepals. For instance a population bred from selected British parents was divided into two groups, (1) plants with two-seeded capsules, and (2) plants with four-seeded capsules. The bract and sepal index values were then noted for each group with the following results: *bract index*, group (1) mean, 1.86 ± 0.0263 ; group (2) 1.67 ± 0.0379 : *sepal index*, group (1) 1.96 ± 0.0329 ; group (2) 1.85 ± 0.0313 .

Crosses were made between plants from America with four-seeded capsules and European plants with two-seeded capsules. With the exception of the occasional occurrence of three-seeded capsules on plants predominantly two-seeded the F_1 populations were two-seeded. An F_2 population of 655 plants gave a ratio of 483:172 of plants with two-seeded capsules:plants with more than two seeds. On the basis of a 3:1 ratio the observed figures agree closely with the expected 491:164.

(6) *Self-fertility.* The American and Greenland sea plantains have proved to be self-fertile in contrast to those of Europe which are self-sterile or are self-fertile only to a slight extent.

IV. THE ANALYSIS OF FERTILITY INTERRELATIONSHIPS OF SAMPLES

Chromosome numbers. In 1934 McCullagh added to the previous list of *Plantago* chromosome numbers the counts for twenty-two species. A subsequent paper was to have included the results of a detailed cytological examination of the material grown at Corstorphine, but the programme was never completed owing to Miss McCullagh's death. She had, however, communicated verbally to the writer her counts for the Greenland sample and for some of the North American and North

European samples. The work was then temporarily undertaken by Miss Bennett of this Institute. The material has now been handed over to Mr Earnshaw of the Edinburgh and East of Scotland College of Agriculture and it is hoped that he will publish an account of his investigations in due course.

These workers have found that the material from North America, Greenland and North Europe has the diploid number 12, which is the number previously recorded for *P. maritima* L. Specimens from the Alpine samples 111, 112, 113, 113 (a), 116 and 118 (see Table I) also have the diploid complement of 12, but in contrast the samples 62, 63, 65, 117, 119 and 130 from the same region proved to be tetraploid with $2n=24$. The alpine region, unlike the others, therefore supports both a diploid and a tetraploid population.

In taxonomic literature three species, *P. maritima* L., *P. alpina* L. and *P. serpentina* All. are recorded as inhabiting this Alpine region. The presence of *P. maritima* L. on the roadside between Mittenwald and Krünn in Bavaria, and *P. serpentina* All. near Scharnitz in Ostmark is reported in the literature: samples 62 from the former habitat and 65 from the latter have both been found to be tetraploid by McCullagh. According to McCullagh (1934) *P. alpina* L. is also tetraploid. Regarding this species Hegi (1906) states that it grows between 1300 and 2250 m. in Bavaria. Collections represented by samples 111, 112, 113 and 113 (a) were accordingly made from this locality at elevations between 1200 and 1700 m. These samples have been found to be diploid by Bennett and Earnshaw. Miss McCullagh had previously very kindly sent the writer her tetraploid specimen of *P. alpina* L. with a note to the effect that it had been obtained from the Royal Botanic Garden, Edinburgh. Enquiries at the Botanic Garden, however, revealed that its source is unknown, although the probability is that it had been obtained from Switzerland. Whether Miss McCullagh's specimen is a garden abnormality of *P. alpina* L. or whether it actually belongs to the Swiss tetraploid population as represented by sample 117 it is difficult to say. What is certain, however, is that in general the Bavarian and Swiss diploids correspond more closely to the accepted description of *P. alpina* L. than do the tetraploids from the same region.

Fertility relationships. In making the artificial hybridizations, self-incompatible female parents lacking the dominant leaf spot were, whenever possible, crossed with males carrying the dominant character, thus obviating the need for emasculation. Emasculation was only resorted to when self-compatible female parents were used or when the leaf spot character provided no check on the success of the cross. All crosses were made in cool greenhouses and the usual precautions relative to artificial pollinations were strictly observed.

The fertility results are presented in Table X. The success of the initial crossings, covering thirty-four out of a possible fifty-five combinations, is recorded in the triangle to the right of the table, while the fertility of some of the F_2 populations is given in the triangle to the left.

In some cases where different parent plants were used to test the fertility of the same combination the crosses were not equally successful. Fertility irregularities were also observed in the F_1 populations. For instance, the F_1 fertility of a cross

West America \times Iceland was considerably lower than the fertility usually exhibited by wild-growing populations, although another F_1 population obtained by crossing the identical American specimen with another specimen from the same Icelandic sample proved to be fully fertile. Therefore, when more than one pair of parents has been employed to test the interfertility of samples of two natural populations, the pair giving the highest seed yield has been taken as the standard of compatibility. When this standard approximates to the seed yield normally obtained for open-pollinated plants of a natural population a + sign has been entered in the table. It

Table X. *Fertility interrelationships*

	Diploids $2n=12$							Tetraploids $2n=24$			
	West America	East America (dense)	East America (lax)	Iceland	Britain	Sweden	Bavaria	<i>P. carinata</i>	Bavaria	Ostmark	(?) Switzerland
West America		+	+	+	+	.	.	.	-	-	.
East America (dense)	.		+	.	.	+	.	+	.	-	±
East America (lax)	.	+		.	.	+	+	.	.	.	±
Iceland	+	.	.		+	+	+	.	.	-	-
Britain	+	.	.	+		+	+	+	-	-	±
Sweden	.	+	±	.	.
Bavaria	.	.	+	.	.	.		+	.	-	-
<i>P. carinata</i>	.	+	.	.	+	.	.		.	-	-
Bavaria		+	+
Ostmark	+		+
(?) Switzerland	+	+	

+

Fertile.

±

Partially fertile.

-

Sterile.

Success of initial cross

F_2 fertility

Success of initial cross

 F_2 fertility+ Fertile. \pm Partially fertile. - Sterile.

will be seen from Table X that the diploid combinations, including *P. carinata* Schrad. (a diploid specimen of this species was obtained from Miss McCullagh), were mutually interfertile. The same is true for the tetraploid combinations. On the other hand when the diploids and tetraploids were intercrossed the results of the pollinations were either negative or only partially successful. The plants raised from the few seeds obtained have been found to be triploid by Bennett and Earnshaw.

In testing the fertility of the F_1 population, six plants of each different combination were spatially isolated and seeded together as a unit. All the F_1 populations representing European combinations or American combinations were fertile. The diploid European \times American combinations were, however, somewhat erratic, relatively low seed production in the F_1 being associated with the partial incompatibility of certain pairs of parents previously mentioned.

F_2 populations were raised from seed collected from the isolated F_1 populations, and six plants of each were similarly isolated. The results are recorded in the left triangle of Table X. It is worthy of note that the population of the combination Western America \times Iceland, which in F_1 had had a decidedly subnormal seed production, had by F_2 considerably increased its fertility.

V. THE SALIENT FEATURES OF THE ANALYSES

1. The North American, Greenlandic and North European samples are diploid ($n=6$). Those from the Alpine region are of two kinds (*a*) diploid, and (*b*) tetraploid ($n=12$).
2. The North American and North European populations, together with the Alpine diploids, constitute one freely intrafertile group while the Alpine tetraploids form another.
3. The North American and Greenlandic plants are self-compatible and have capsules which are typically four-seeded. In contrast the European plants are self-incompatible, or almost so, and have capsules which are typically two-seeded. These characters exhibit the only abrupt transition which has been observed between the North American and North European populations.
4. The variate ranges for the measurable characters of all subregions overlap.
5. The bracts and sepals of the American samples are, on the average, relatively broader in proportion to length than those of North Europe.
6. The subregional mean values of the bract and sepal indices and of the ratio scape length:spike length follow a geographical gradient with low values in western North America rising through eastern North America, Iceland, Faroes and Britain to maxima in Sweden and falling again in South Germany.
7. The subregional values for the character leaf pubescence (see Table V) follow a geographical gradient, being relatively low in western North America, rising in eastern North America and attaining a maximum in Iceland, thereafter falling steadily through Faroes and Britain, and reaching a minimum in South Sweden, only to rise again in South Germany.
8. The leaf-spot character which occurs commonly in samples from North Europe, Greenland and eastern North America has not been recorded in samples from the Pacific coast or from the Alpine region. The gene responsible for the expression of this character is therefore present in both continents but is not universally distributed in either. The subregional frequency percentages (Table VIII) form a semblance of a geographical gradient with low values in the Faroes and Britain and high values in northern North Europe, Greenland and eastern North America. Locally, however, the frequency values are peculiarly irregular.

9. In eastern North America spike density follows an ecological gradient. The change from rock habitats to salt-marsh habitats is accompanied by a change from relatively dense spikes to relatively lax spikes. The presence of a dominant gene in this subregion is apparently responsible for the expression of the lax-spike character. In Europe the exact equivalent of the lax gene is probably absent, which may account for the lack of such clear-cut differentiation into lax- and dense-spiked populations in this region. Nevertheless, in Britain there is a tendency for the sea-shore samples to have slightly lower spike density values (12.54 flowers per cm.) than the inland samples (13.97 per cm.). This difference, however, seems to be at least partly due to the fact that the coastal samples have, on the average, longer spikes than the inland, a weak inverse correlation occurring between spike length and spike density (Davey & Lang, 1939).

10. Associated with the eastern North American ecological gradient for spike density is a gradient for sepal index. The sepals of the rock samples are narrower in relation to their length than those of the salt-marsh samples.

11. In Britain growth habit and scape length follow an ecological gradient (Gregor, 1938). In the coastal areas growth habit becomes progressively more erect and the scapes become progressively longer as the habitat conditions change from waterlogged mud to drained mud.

VI. CLASSIFICATION

The units of orthodox taxonomy. The diploid sea plantains of Europe and North America which have been treated in this paper are represented taxonomically by at least five species—*Plantago maritima*, L., *P. alpina* L., *P. carinata* Schrad., *P. juncoides* Lam., and *P. oliganthos* R. & S. From the literature it would appear that *P. maritima* L. is the most widespread species, having been recorded from North America as well as from Europe. But Fernald (1925) has drawn attention to the doubt in the minds of many American botanists as to the occurrence of this species in North America. He concludes that North America is represented by two species, *P. juncoides* Lam. (including *P. decipiens* Barnéoud and *P. borealis* Lange) and *P. oliganthos* R. & S. On p. 93 Fernald writes "whether true *P. maritima* occurs in America is not wholly clear. In the Gray Herbarium there is a specimen typical in every detail marked Sitka (coll. Bongard) but all other Alaskan material seen is Pacific American *P. juncoides* Lam."

The characters employed by Fernald to distinguish *P. maritima* of Europe from the sea plantains of North America are (1) higher bract and sepal indices, (2) greater ciliation of the sepals, (3) more slender capsules, (4) greater anther length, and (5) a higher scape length: leaf length ratio.

Although no significant break in the range of sample mean values occurs for the bract and sepal indices between North America and North Europe, the indices are, on the average, significantly lower in North America than in Europe. But the broader sepals of North American populations are not invariably less ciliate than the sepals of European populations with higher bract index values. In fact the sepal margins and keels of sample 89 from Atlantic America (bract index mean 1.86) were

more ciliate than those of the British sample 21, the sample with the highest bract index mean value (2.25) in that subregion. No correlation has been found between the bract or sepal indices and anther length (Davey & Lang, 1939). It is, therefore, not surprising to find that samples with the same anther length differ widely in respect of their bract and sepal indices. For instance, sample 39 from Britain with an anther length of 1.80 mm., and sample 60 from Nova Scotia with the value 1.79 mm., have respectively sepal indices of 2.33 and 1.61. And finally, while a high scape length:leaf length ratio is reputed to be diagnostic of *P. maritima* of Europe, the ratio of 1.18 for the British sample 35 is lower than any recorded for the Atlantic American samples.

Fernald (p. 95) states that: "In the northern half of its range *P. decipiens* becomes very dwarfed, with scapes only 1-7 cm. long and spikes 0.5-2 cm. long. This is the plant of Greenland, Iceland and Arctic Europe described and beautifully illustrated by Lange." According to Ostenfeld & Grøntved (1934) *P. maritima* also occurs in Iceland, and therefore a critical examination of the Icelandic samples became necessary. If both these authors are correct then the ranges of *P. juncoides* Lam. and *P. maritima* L. are coincident in this area, a circumstance which might account for the lack of discontinuity in respect of the bract and sepal indices between the North American and North European populations. But an examination of the character mean values and more particularly of the variate ranges of the seventeen samples from Iceland gives no support to the contention that Iceland is inhabited by two distinctive populations; neither is this Icelandic population distinct from those of the Faroes and of Britain. If a specific distinction between the North American and North European populations were to be made at all it would be more appropriate to draw the line between Greenland and Iceland than to postulate the existence of a North American species in Iceland, for it is between these two countries that the only marked character changes occur, viz. the changes from four-seeded to two-seeded capsules, and from self-compatibility to self-incompatibility.

Samples 84 and 81 represent respectively *P. juncoides* Lam. and the eastern American species *P. oliganthos* R. & S., and as they were collected by Mr Weatherby of the Gray Herbarium they can be regarded as authentic samples. The characters attributed to the species *P. juncoides* and *P. oliganthos* have been found to vary quantitatively and, while a minority of variates in a sample may exhibit characters conforming to the specific type, the majority have characters which can be found in some proportion in all samples. In the case of samples 84 and 81 only a relatively small number of the variates possessed by the latter have characters peculiar to that sample. For instance, of the total constituents of sample 81, 21% were found to have sepal margins less ciliolate than the least ciliolate variates of 84, 10% had capsule apices less ciliolate, 10% had larger seeds, 9% had a lower scape length:leaf length ratio, and 7% had spikes more lax than the least densely spiked variates of 84. None had bract margins and keels less ciliolate, nor did any have thicker leaves than variates in 84. These characters are diagnostic of the two species, but, as the above treatment shows, the difference between 81 and 84 is far less dependent on character combinations peculiar to one or the other than on differential frequencies of indi-

vidual characters common to both samples. An estimate of the extent by which populations differ from each other can be obtained by employing the *aggregate difference* method described and used by Anderson & Abbe (1934, p. 47). Provided the same characters, measured on the same scale, are utilized in making comparisons the aggregate difference gives an indication of the magnitude of population divergence. For comparative purposes the two samples 81 and 84 have again been used, together with sample 89. This latter sample is also from eastern America and in appearance resembled 84 more than it did 81, a general observation which is given more precise expression below. In respect of the mean values for the characters of the spikes, bracts, sepals, seeds, anthers, capsules and leaves (twenty-two characters in all) the aggregate difference between samples 81 and 84 has been found to be 4.45; between 84 and 89 the index is 4.18; while between 81 and 89 it is 6.84. As far as

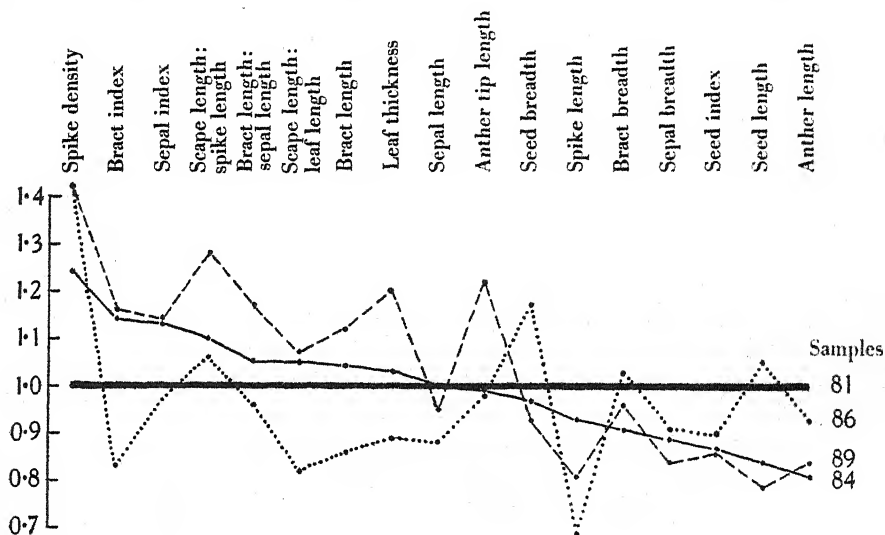


Fig. 2. The relative position of sample 81.

the individual characters which contribute to those indices are concerned sample 84 also occupies an approximately intermediate position between 81 and 89. Thus, while both 84 and 89 differ quantitatively from 81 by a group of characters, the greatest divergence is exhibited by 89. It might, therefore, be considered that herein lies the distinction between *P. oliganthos*, as represented by 81, and *P. juncooides*. But this is not the case since many of the characters distinguishing 81 from 84 and 89 are not the ones distinguishing 81 from *P. juncooides* in other parts of its range. For example, if the individual mean values for the measurable characters of spikes, bracts, etc., of sample 81 are taken as unity, and if the mean values for the same characters of samples 84, 89 and a western American sample 86 are given their proportionate value above or below this standard, it will be seen (Fig. 2) that, in the aggregate, the difference between 81 and 89 is actually less than the difference between 89 and 86, a sample from an area where *P. juncooides* alone grows. In fact

it would not be difficult to find individual specimens outside the eastern American subregion which could with some justification be referred to *P. oliganthos* R. & S. Similarly, some of the rare plants with four-seeded capsules which occur in widely separated European localities might easily be confused with *P. juncoides* Lam.

Too great a reliance on specific descriptions may lead to the recording of distributional anomalies. For example, Whyte (1869) has recorded the presence of three species, *P. maritima* L., *P. alpina* L., and *P. serpentina* Vill. from the Scottish mountains, while Hegi (1906) records the occurrence, though as uncertain, of *P. alpina* in Iceland. There is no doubt that a few plants conforming to the description of *P. alpina* are to be found not only in Britain and Iceland but also in the Faroes. However, there seems to be no reason for assuming that such exceptional variates represent a population within a population. More pertinent are speculations as to whether *P. maritima* occurs in the alpine region, because in this region there occur tetraploid populations containing many variates which cannot be distinguished morphologically from diploid variates belonging to British and Swedish samples, e.g. the tetraploid population (sample 62) on the roadside between Mittenwald and Krünn in Bavaria.

The units of experimental taxonomy. A disadvantage of the present system of classification as far as the experimentalist is concerned is that the taxonomic units do not always coincide with the categories of variation. The Mittenwald population is a case in point. Unfortunately this objection is inherent in a system in which the units are founded on a complex of attributes irrespective of their individual variational significance. If, instead, the attributes of populations were to receive individual treatment it would then be possible to record by means of the same notation both the *variational type* and the taxonomic status of populations exhibiting these separate attributes. The potential ability of members of a population freely to exchange genes would seem to be a fundamental "specific" criterion of variational significance. From the result of hybridization experiments it has been seen (Table X) that, when given an opportunity to hybridize, the North American-North European-diploid Alpine population (comprising *P. juncoides* Lam. (including *P. decipiens* Barnéoud and *P. borealis* Lange), *P. oliganthos* R. & S., *P. maritima* L., *P. alpina* L., and probably *P. carinata* Schrad.) constitutes a single intrafertile group, although in such a widespread population the potential gene exchange is not realized in nature owing to the spatial isolation of its parts. But as the adoption of the criterion of gene exchange would involve a redefinition of the taxonomic term species it is proposed to avoid this term in a complementary system of classification and employ in preference the term *Coenospecies* (Turesson, 1922) for a group of sexually reproducing plants separated from other groups by sterility or by failure of the hybrids to produce viable seeds. The coenospecies is then "that stage of evolutionary divergence at which the once actually or potentially interbreeding array of forms becomes segregated in two or more separate arrays which are physiologically incapable of interbreeding" (Dobzhansky, 1935, p. 354). A coenospecies is more likely to represent a Linnean species or group of Linnean species than a population within such a species, but whatever the range of morphological variation the criterion would be its inability

to exchange genes with other populations, even when given the opportunity of so doing. All parts of the diploid sea plantain population belonging to the *P. maritima* group would, therefore, belong to the same coenospecies, i.e. to the same category of variation. The inclusion of the alpine tetraploids in this coenospecies must await proof of gene exchange between them and the diploids. Proof that these tetraploids and diploids can be crossed has already been obtained.

"Species arrange themselves in natural groups because some of them have a slight [low] capacity for exchanging genes" (Clausen, 1936, p. 522). Units of this order within a coenospecies have been termed *ecospecies* (Turesson, 1922), one ecospecies being separated from another by restricted interfertility and by failure of hybrids to establish themselves in nature. Clausen (p. 522) believes that "ecospecies should be regarded as the taxonomic species, because they represent the smallest units which keep separate by the aid of an inner balance mechanism". The maintenance of their individuality he regards as being due to the destruction of hybrids under natural conditions "and the few vigorous offspring left will largely drop back into one or other of the two original species, sometimes adding one or a few genes from the other, thus increasing their variability and creating the phenomenon of parallel variation as described by N. J. Vavilov".

Since no inherent restriction is imposed on the free exchange of genes between parts of the diploid plantain population and since no lack of vigour has been observed in the F_2 generations, all the diploids belong to the same ecospecies. If it should be found that the diploid and tetraploid groups are capable of exchanging genes, then they would belong to different ecospecies of the same coenospecies, if not, the two groups would belong to separate coenospecies.

Now when dealing with morphologically differentiated populations, which are physiologically capable of freely interbreeding, i.e. populations within an ecospecies, it is necessary to consider their permanency as distinctive morphological units. To describe a population in terms of a peculiar combination of characters and allocate populations to different categories on the basis of the observed degrees of difference between combinations implies that for taxonomic purposes such combinations bear a static relationship to one another. But all evidence favours the view that few populations, especially the smaller breeding communities, are morphologically stable, even temporarily. The gene complexes of small sea plantain communities are ever susceptible to change resulting from the periodic attacks of grazing animals, the partial or complete depopulation of existing habitats by flood action, and the repopulation of old and the colonization of new habitats. Even the larger communities are not immune from the effects of environmental fluctuations such as climatic variations. Elton's (1924) work on the fluctuations in animal numbers suggests that short-period pulsations of climate operating over wide areas affect the genotypes of large populations in as much as "every time a minimum in numbers occurs, there is a chance of the less common genes becoming extinct" (p. 159). On the other hand, when recolonization is in progress the chance of any individual surviving is greatly increased. It follows, therefore, that an inventory, if that were possible, of every morphologically discrete population, i.e. one possessing its own peculiar range of

morphological variation, would be no sooner completed than it would be out of date.

It would, nevertheless, be a mistake on the part of taxonomists to ignore altogether minor populations, e.g. breeding communities, on account of their instability, because they just as surely represent links in an evolutionary network as do the more obvious units of higher taxonomic rank. But the problem of recording population differentiation is further complicated by the fact that when spatial isolation is less than absolute, breeding populations must to some extent interbreed with their neighbours, and thus gene exchange between breeding communities has also to be considered. If, therefore, instead of attempting to describe a multitude of populations in terms of their discontinuities in character complexes, taxonomic emphasis were to be transferred to recording the populations typifying particular kinds of variation, e.g. geographic, ecologic, etc., regardless of the actual degree of aggregate morphological distinctness exhibited by individual populations, then the problem would be simplified into one of studying individual "indicator" characters (in contrast to complexes of characters). That is, from the taxonomic standpoint, inherent morphological and physiological attributes of populations would be used mainly as indicators of the natural arrangement of populations in response to factors external to the plants themselves. In this way the discontinuities, proportional representations and trends in the distribution of individual characters and their variations could be assessed in terms of populations having either discrete or overlapping distributions. For example, the leaf-spot character previously mentioned is an "indicator" carried by a geographical population covering northern Europe and eastern America; its local absence within this area is indicative of a chance fractionation of a population followed by spatial isolation. The European portion of this leaf-spot population lies within a two-seeded capsule population, while the American part lies within a four-seeded capsule population. Within Britain the three growth-habit categories, decumbent, ascending and erect, have been used (Gregor, 1938) as indicators of the ecological relationships of populations.

A form of differentiation within the ecospecies is when a character's expression tends to change quantitatively and progressively from one place to another. The variations of the characters bract index, sepal index, scape length:spike length ratio, and leaf pubescence exhibit this particular kind of geographical relationship in as much as when their subregional mean values are treated in geographical sequence the variation in character expression follows a geographical gradient. These gradients, however, are not coincident, the characters varying independently of each other in different geographical directions. This is shown in Fig. 3 where the bract index, sepal index, scape length : spike length ratio, and leaf pubescence gradients are represented graphically by connecting the subregional character mean values. The vertical lines represent the subregional variate ranges while the small horizontal lines mark the extremes of the range of the sample mean values. In the case of the leaf-spot character, local differences in the frequency of spotted plants, differences which are apparently due to chance, are very pronounced and make it impossible to demonstrate even a semblance of a geographical gradient within subregions. But

the average subregional percentage frequencies do tend to follow a geographical sequence (Fig. 4).

Another kind of gradient in character expression which, unlike the above, does not necessarily follow a geographical sequence is exemplified by the characters

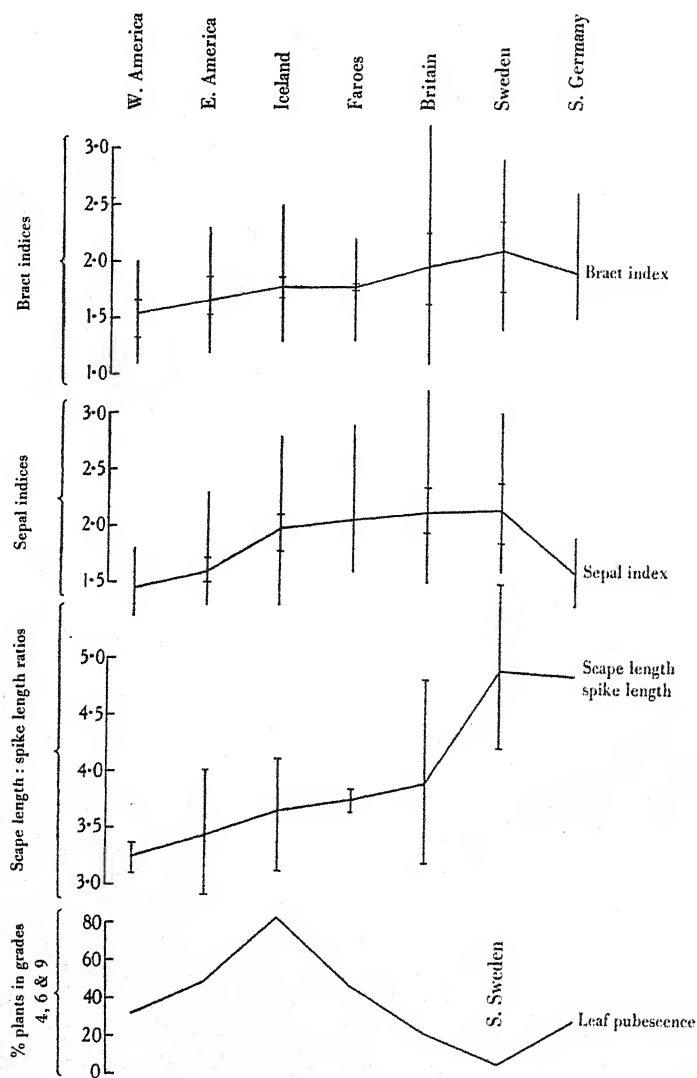


Fig. 3. Bract index, sepal index, scape length: spike length and leaf pubescence. Topoclines.

growth habit and spike density. In Britain the change from waterlogged to drained coastal habitats is accompanied by an increase in scape length and a more erect growth habit (see Gregor, 1938). No geographical gradient in respect of these characters has been established for Britain, nor was it likely that such a gradient

would be found owing to the irregular distribution of the different kinds of habitat. Locally, the environmental change from one habitat to another may be considerable and abrupt, but taking the British subregion as a whole the variation between the one extreme and the other is continuous. Corresponding to the habitat continuity, a continuous range in sample mean values has been found for scape length and growth habit (Fig. 5). In much the same way spike density in eastern America follows an ecological gradient from salt-marsh to maritime rock habitats (Fig. 5).

Variation which follows either a geographical or ecological gradient may be said to be *clinal*, and populations possessing characters which behave in this manner can be recorded under the taxonomic title *cline* (Huxley, 1938). A cline is defined as *any gradation in measurable characters*. In the present paper only those gradations in phenotypic characters which are regarded as having a genetic basis will be considered. Prefixes can be used to denote clines of different types, for example *topocline* (geographical cline), *ecocline* (ecological cline). The recognition of an ecocline involves

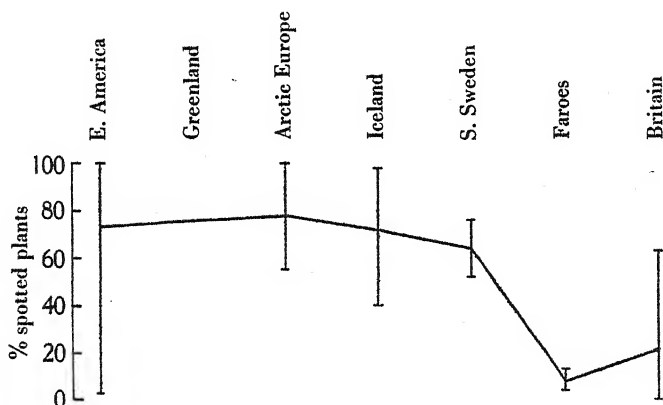


Fig. 4. Leaf-spot. Topocline.

the appreciation of its correlation with an observable gradient in environmental conditions, for example the scape length ecocline of the British sea plantains is apparently adapted to edaphic variations. The topocline, on the other hand, is a character gradient tracing an essentially geographical course, but in all probability reflecting the effects of its past as well as of its present environmental contacts. An example of a topocline is the progressive change in the proportions of the floral bracts from western America to northern Europe. That the influence of the prevailing environment on a topocline may be indirect is suggested by the fact that, in individual breeding communities, scape length (a character indicative of plant size) is correlated in varying degrees with the lengths and breadths of both bracts and sepals (Davey & Lang, 1939, p. 19). The, at least partial, interdependence of floral proportions on scape length is further demonstrated by the sample mean values in respect of scape length being correlated with the bract index and sepal index means, the respective values of r being $+0.64$ and $+0.32$. However, the regression coefficients show that bract index rises by 0.014 and the sepal index by only 0.007 for every increase of

1 cm. in scape length. Therefore, in terms of index values the general effect of scape length on bract and sepal proportions, significant only in the case of the bracts, is not sufficiently pronounced to obscure the local operation of contributory factors

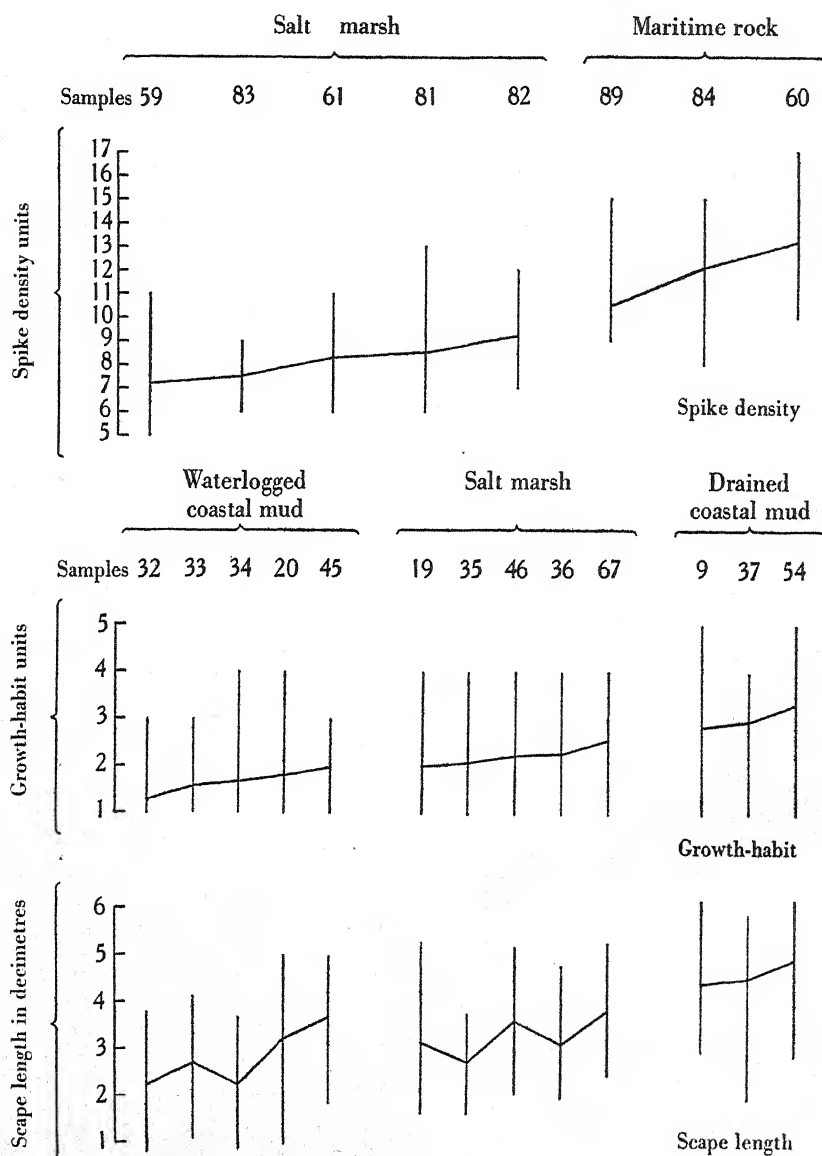


Fig. 5. Spike density and growth-habit. Ecoclines.

such as differences in the number of seeds per capsule and the differences in spike density; the latter character is sometimes correlated with the bract and sepal dimensions, independently of scape length (Davey & Lang, 1939, p. 7). It follows then

that the bract and sepal index topoclines, while exhibiting no apparent coincidence with perceptible environmental gradients over their whole distributions, must in any given locality be seldom, if ever, entirely free of the influence of the prevailing environment acting indirectly through the medium of associated characters.

Treatment by clines thus provides a method of recording separately the various geographical and ecological trends in character expression, while a collective examination of clines makes it possible to trace the local relationships of character combinations. For example, the combination low sepal index-low spike density is a characteristic of the salt-marsh samples of eastern America, but the same sepal index is, in other parts of the American Continent, associated with a relatively high spike density, e.g. in Alaska (sample 86).

As previously mentioned, however, abrupt character changes which follow no clinal sequence occur and differentiate parts of a large population. Such differentiation may be due to the presence or absence of one component of a contrasting character pair. For instance, the eastern American-northern European population possesses a leaf-spotting gene which is apparently absent from Pacific America and the Alpine region. Differentiation of this abrupt kind may be said to be *extraclinal*. On the other hand, it may be necessary to refer to particular ranges (distributions) on a topocline, e.g. to the Icelandic range of the leaf pubescence cline or to the North American range of the sepal index cline, etc. Such population differences are *intraclinal*. It is proposed to apply the term *topotype* to both extra- and intraclinal geographical variation and to define a topotype as follows: *a population in a geographical region possessing characters differing from those of another region*. A topotype may be *extraclinal* if it does not fall within a geographical gradient in phenotypic characters, or *intraclinal* when it refers to a particular range within a geographical character gradient. The term *goetype* has been used by Remane (1928, pp. 65, 75) to denote a geographical race and therefore the term as defined above is not synonymous.

It will also sometimes be necessary to make reference to populations within an ecocline. Populations which occupy particular ranges on an ecocline are *ecotypes* (Turesson, 1922), e.g. the British populations exhibiting a predominating frequency of ascending variates belong to the "ascending" ecotype of the "growth-habit" cline. It should perhaps be emphasized that the categories of topotype and ecotype are population concepts, not morphological classes. That is to say, a decumbent individual from a population classified as an ascending ecotype would, despite the fact of its being decumbent, belong to the ascending ecotype, and not to a taxonomic category comprising only decumbent variates.

A considerable amount of local differentiation may occur between isolated populations due primarily to the chance fixation of characters of no ecological significance or, at most, of wide environmental tolerance. For example, in Britain (i.e. within the distribution area of the leaf-spot topotype) isolated populations lacking the leaf-spot gene are to be found. Such differentiation is probably partly due to predetermined variation and partly to subsequent random variations in the gene frequencies (see Wright, 1932; and Dobzhansky, 1937, pp. 134 *et seq.*).

Populations locally differentiated, but lacking apparent adaptive significance, could be referred to as *microtopotypes* which may be defined as *locally differentiated populations which may occupy similar habitats, and which are considered to have arisen by the chance fractionation of a parent population*. A microtopotype, then, is a micro-geographical population whose individuality is recorded in terms of an indicator character which, though tolerant of the prevailing environment, is not perceptibly correlated with any particular kind of habitat.

The above terms have reference only to population characteristics but it not infrequently happens that striking variates, e.g. golden chlorophyll-deficient plants, plants with leaf-like bracts, etc., occur so rarely and so sporadically that they never become a feature of any population or group of populations. For genetic, economic or other reasons it may sometimes be necessary to record the occurrence of these exceptional hereditary aberrations, and the term *exotype* (Remane, 1928) would seem to be an appropriate one to use in this connexion.

The application of reference names. The accepted bi- and trinomial nomenclature would apply to the respective "species" units—the coenospecies and ecospecies. But populations accredited to the various geographical and ecological categories would require nothing more than descriptive symbols, and in most cases the name of the distinguishing character, or some suitable abbreviation, would be sufficient designation. For example, the description "immaculate" might conveniently be given to the toptype lacking the leaf-spot gene; this abbreviation would of course also apply to the microtopotype which lacks the same gene. These populations under their respective categories could then be presented in list form below their appropriate coenospecies or ecospecies. By this arrangement the spatial associations of characters, e.g. of toptypic and ecotypic characters, could be assessed at a glance. Any attempt to apply an extension of the trinomial system to such populations would not only be confusing but misplaced, as it might suggest a progressive subordination of categories which has never been implied. A classification of the diploid sea plantains of North America and Europe based on the available data and incorporating the foregoing suggestions can be summarized as follows:

COENOSPECIES: *Plantago coeno-maritima* including the diploid populations of *P. maritima* L., *P. alpina* L., (?) *P. carinata* Schrad., *P. juncoides* Lam., *P. decipiens* Barnéoud, *P. borealis* Lange, and *P. oliganthos* R. & S.

ECOSPECIES: *P. coeno-maritima eco-maritima* (see p. 314).

TOPOTYPES (extraclinal):

- (a) *Self-compatible, capsules typically four-seeded.*
North America and Greenland.
- (b) *Self-incompatible, capsules typically two-seeded.*
Europe.
- (c) *Leaf-spot presence* ("maculate").
North Europe, Greenland and eastern North America.
- (d) *Leaf-spot absence* ("immaculate").
Western North America and Alps.

CLINES:

- (a) *Bract index*, topocline (see Fig. 3):

TOPOTYPES (intraclinal): (1) *North American*, mean = 1.61, range of sample means = 1.33–1.86; (2) *North European*, 1.93, 1.62–2.35.

- (b) *Sepal index*, topocline (see Fig. 3):

TOPOTYPES (intraclinal): (1) *North American*, 1.58, 1.45–1.71; (2) *North European*, 1.93, 1.78–2.38.

- (c) *Scape length : spike length ratio*, topocline (see Fig. 3).

- (d) *Leaf pubescence*, topocline (see Fig. 3).

- (e) *Leaf-spot*, topocline (see Fig. 4).

MICROTOTYPES: (1) "maculate" (100 % spotted); (2) "immaculate" (0 % spotted).

- (f) *Spike density*, ecocline (see Fig. 5).

- (g) *Growth habit*, ecocline (see Fig. 5).

ECOTYPES: (1) "decumbent"; (2) "ascending"; (3) "erect".

EXOTYPES:

- (a) "Golden" (a chlorophyll deficient).

VII. SUMMARY

1. It is suggested that experimental taxonomists should in the meantime refrain from attempting to meet their requirements by redefining the orthodox classificatory categories, but should, instead, use a complementary system of classification with a distinctive terminology.

2. It is also suggested that taxonomic emphasis might be usefully transferred from morphological character complexes to individual morphological and physiological attributes indicative of particular kinds of differentiation.

3. The use of the following "specific" terms is advocated: *Coenospecies*, a population which is incapable of exchanging genes with other populations, even when given the opportunity. *Ecospecies*, a population with an inherently low capacity for exchanging genes with other populations of its coenospecies.

4. The use of the following "infra-specific" terms is advocated for populations actually or potentially capable of freely exchanging genes:

Cline, any gradation in measurable characters.

Topocline, a cline following a geographical gradient.

Ecocline, a cline apparently correlated with an observable ecological gradient.

Topotype, a population in a geographical region possessing characters differing from those of another region. A topotype may be *extraclinal* if it does not fall within a geographical gradient in character expression, or *intraclinal* if it has reference to a particular range on a geographical gradient.

Ecotype, a particular range on an ecocline.

Microtopotype,* a micro-geographical population primarily the result of a chance fractionation of a parent population.

Exotype, an hereditary aberration which occurs so rarely and so sporadically that it never becomes a feature of any population: the category of exotype is therefore not a population concept.

I am much indebted to those biologists whose names appear in Table I for their generous help in collecting material, and to Mr J. S. L. Gilmour and Dr Julian Huxley for constructive criticism.

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* I have substituted *microtopotype* for the term *geocotype* proposed by me in 1931. In the terminology of type specimens *topotype* already appears and means a specimen from the same locality as the type. It is unlikely, however, that confusion will arise by the double use of the term as the contexts in which they will appear will always make the meaning clear.

AN ASYNAPTIC OENOTHERA

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(With 18 figures in the text)

IN the course of a study of half-mutants from *Oenothera Lamarckiana*, a strain of *erythrina* has been found in which the segregated *decipiens* individuals show no chromosome pairing at diakinesis and metaphase I of meiosis. This situation is commonly designated "asynaptic".

Prof. T. J. Stomps, of Amsterdam, who very kindly gave me seeds, states that this strain of *erythrina* arose in 1924, from selfed *Lamarckiana*, that is, independently of the strains studied by de Vries (1919). It behaves like the strains of *erythrina* examined by de Vries in regularly segregating the homozygous type *decipiens*. Both the half mutant and the full mutant agree well with the descriptions of Prof. de Vries, and in fact the latter believed Prof. Stomps' strain to be true *erythrina*. The following is mainly an account of the cytological situation in these plants, together with data contributing to the location of the gene concerned.

MATERIAL AND METHODS

A family of thirty-three plants was grown in 1936 from selfed seed supplied by Prof. T. J. Stomps. It contained fourteen *erythrina* and nineteen *decipiens* plants. All the *erythrina* plants had dehiscent anthers and abundant pollen. All the *decipiens* plants had thin indehiscent anthers and scanty pollen; three plants tested were very highly sterile in outcrosses both as female and male parents. Three plants of each type were examined cytologically, partly by means of permanent smears of pollen mother cells and partly by means of buds fixed whole, sectioned at 20μ in paraffin wax and stained with gentian violet following Smith's (1934) picric acid technique. Both smears and whole buds were fixed in Belling's modification of Navashin's fluid. The chromosomes in the whole bud fixations were rather more swollen than those in the smears, probably owing to more rapid penetration of the acetic acid constituent of the fixing fluid. The drawings were made with the aid of a camera lucida, using a Zeiss 1.4 n.a. 2 mm. objective and a $\times 20$ ocular; their magnification is 3000.

CYTOLOGICAL DESCRIPTIONS

O. erythrina has a ring of six chromosomes and four pairs at diakinesis and metaphase I of meiosis (Figs. 1, 2). The ring and the bivalent chromosomes are subject to the usual failures of chiasma formation and the ring of six is also sometimes non-disjunctional.

O. decipiens usually shows fourteen univalents at diakinesis and metaphase I in the pollen mother cells, but sometimes one or more bivalents and consequently fewer univalents. The first observations, based on several rather indifferent smears



Figs. 1-2. Polar and side views of metaphase I chromosomes in pollen mother cells of *Oenothera erythrina*, showing a ring of six chromosomes and four pairs.

from the same plant, showed out of fifty-one pollen mother cells at metaphase I only two with a single rod bivalent each; the remaining forty-nine cells each had fourteen univalents. Later studies showed such a high failure of pairing to be exceptional. The following description refers exclusively to the course of meiosis in *decipiens*.

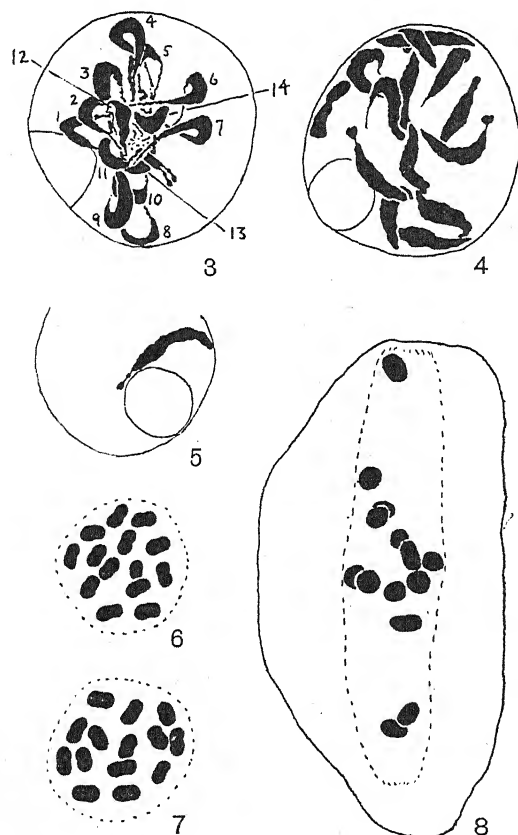
Nothing very definite can be made out in the pollen mother cells until diakinesis is approached. Like the normal synaptic diploid, the prophase nucleus contains a balled mass of chromosome threads attached to a single nucleolus. The pachytene chromosome threads do not appear to be thinner than those of the normal strain at a comparable stage, but in both cases it is equally impossible to be certain that the threads are double. At a stage corresponding to mid-diplotene (Fig. 3) there is a rather dense mass of tangled chromosomes, usually with some thick loops projecting from it. Occasionally, as in the nucleus illustrated, it is possible to count fourteen such loops. The loop is very thick at its bent portion, but thins out rapidly to a slender thread as it approaches the central tangle. Comparison with later stages shows that each loop represents the precociously condensed middle region of a chromosome. The fourteen loops thus account for fourteen chromosome middles; the twenty-eight uncondensed ends cannot be followed in the tangle. Precocious condensation of the middle regions seems to be a characteristic of *Oenothera* chromosomes; perhaps these parts are relatively inert and heterochromatic in the sense of Heitz.

At late diplotene, apart from cases of terminal or interstitial chiasmata, the chromosomes are quite unassociated. They are like diakinesis chromosomes except that the ends are drawn out into long threads. No case of coiling of one chromosome about another has been observed.

At diakinesis, the degree of contraction of the chromosomes is identical with that in normal diploids. Most nuclei at this stage show fourteen univalents (Fig. 4), some one or more bivalents. The chromosomes are commonly grouped to one side of the nucleus; this polarization is a relic of the earlier prophase condition. In some diakinesis nuclei one or two chromosomes may be seen attached to the nucleolus. This attachment (Fig. 5) is intercalary and is apparently proximal to a rather long, faintly staining constriction on which lies a small, sharply staining, granule; distally there is a small trabant. Usually the nucleolar chromosomes are detached from the nucleolus before this stage, though two chromosomes with trabants are commonly visible.

At metaphase I, the chromosomes are fully contracted, and easily distinguishable from one another. Their degree of contraction is equal to that of *O. erythrina* chromosomes; compare, for instance, the polar views of metaphase I (Figs. 6, 7) with the polar views in *erythrina* (Fig. 1).

The range of pairing types, with their observed frequencies and references to figures, is given in Table I. This records an analysis of 1582 pollen mother cells, at metaphase I, seen in sections of one bud. Other buds showed similar kinds of

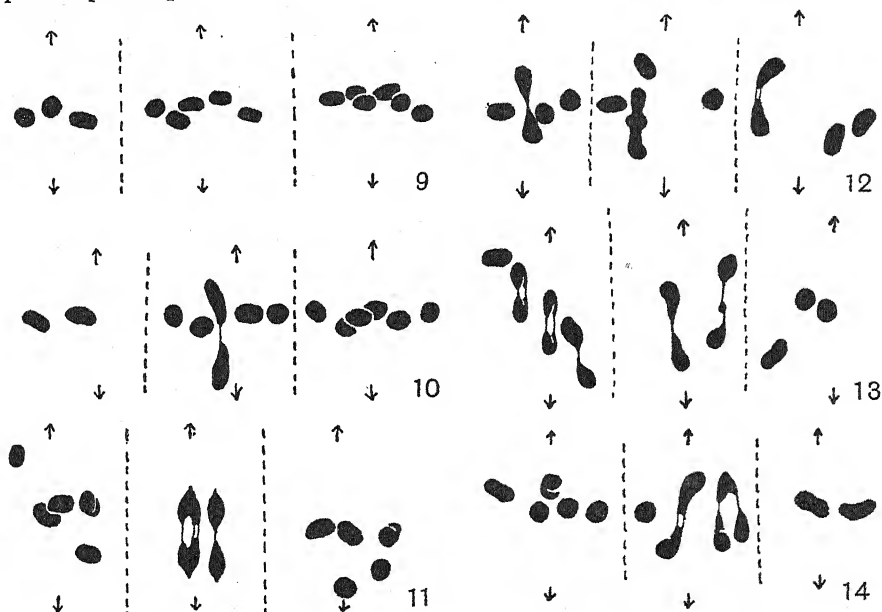


Figs. 3-8. *Oenothera decipiens*. Fig. 3. Diplotene. Figs. 4-5. Diakinesis. Figs. 6-7. Polar views of metaphase I. Fig. 8. Side view of early metaphase I showing scattered univalents.

chromosome pairing. Notable are the two cases of chain trivalents (Fig. 14) which are exceptional on the supposition that *decipiens* is homozygous.

The numbers of chiasmata observed per pollen mother cell range from 0 to 7, the mean number being 0.68. The total number of potential bivalents in the 1582 cells is 11,074. Of the 949 bivalents observed, 120 had chiasmata in both arms; the random number would be 81.3. The relatively high frequency of bivalents with two chiasmata suggests that the occurrence of one chiasma in a bivalent raises the chance of occurrence of a second one in the same bivalent.

At metaphase I the chromosomes are normally more or less closely grouped about the equator of the spindle. Rarely they may be scattered more or less from pole to pole (Fig. 8); but such cells may represent stages in metaphase congression. The bivalents are regularly oriented with their two chromosomes directed towards opposite spindle poles. The univalents (cf. Figs. 6-14) nearly always lie with their



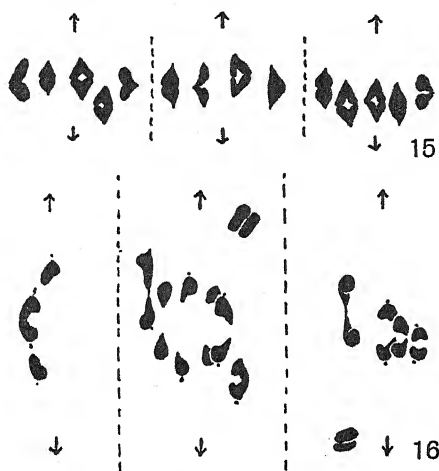
Figs. 9-14. *Oenothera decipiens*. Side views of metaphase I chromosomes in pollen mother cells drawn in three foci. The arrows indicate the longitudinal axis of the spindle. See text and Table I for descriptions.

Table I. *Metaphase I configurations in Oenothera decipiens pollen mother cells, with their absolute frequencies and chiasma frequencies*

Configuration	Number of pollen mother cells	Chiasma frequency per pollen mother cell	Text-figure no.
14 univalents	890	0	6, 7, 8, 9
1 rod bivalent, 12 univalents	420	1	10
1 ring bivalent, 12 univalents	62	2	—
2 rod bivalents, 10 univalents	135	2	—
1 ring bivalent, 1 rod bivalent, 10 univalents	31	3	11
2 ring bivalents, 10 univalents	2	4	—
3 rod bivalents, 8 univalents	22	3	12
1 ring bivalent, 2 rod bivalents, 8 univalents	10	4	—
2 ring bivalents, 1 rod bivalent, 8 univalents	2	5	—
3 ring bivalents, 8 univalents	1	6	—
4 rod bivalents, 6 univalents	2	4	—
1 ring bivalent, 3 rod bivalents, 6 univalents	1	5	—
2 ring bivalents, 2 rod bivalents, 6 univalents	1	6	—
2 ring bivalents, 3 rod bivalents, 4 univalents	1	7	13
1 chain trivalent, 11 univalents	1	2	—
1 chain trivalent, 1 rod bivalent, 9 univalents	1	4	14
	1582		

long axes transverse to the long axis of the spindle. The univalents rather often, but not always, form an almost flat plate across the equator of the spindle.

At anaphase I, the two chromosomes of a bivalent disjoin towards opposite spindle poles. Then the split in the univalents becomes evident and their two daughter chromatids separate and move towards opposite poles. This division seems to be accomplished in all univalents at anaphase I whether or not they are precisely on the equator of the spindle (Figs. 15, 16). Even when a univalent is some distance



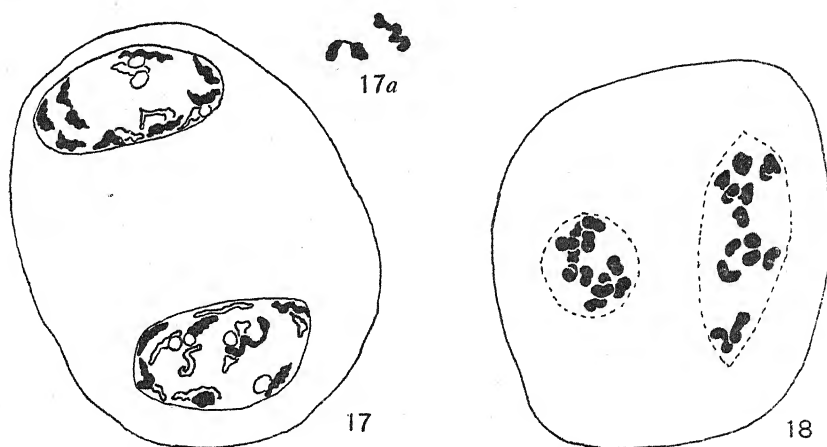
Figs. 15-16. *Oenothera decipiens*. Side views of anaphase I chromosomes in pollen mother cells drawn in three foci. Fig. 15 shows an early stage in division of fourteen univalents; Fig. 16 shows a later stage in division of twelve univalents which has started after separation of the chromosomes of a bivalent. Centromeres are shown in the cell from which Fig. 16 was drawn.

off the equator towards one pole, it divides and the products of the division disjoin towards opposite poles. In Fig. 16, for example, one bivalent has disjoined well in advance of the division of the univalents and the split in the chromosomes that constituted the bivalent, and which are now at the poles, has become evident. The progress of the division of the univalents, also, is unaffected by their situation with respect to the equator of the spindle. Those off the equator in Fig. 16 are as far advanced with their division as those on the equator.

As a result of this regular division of the univalents at division I, two interphase nuclei are always formed. Presumably, even cells with the univalents as scattered as those in Fig. 8 pass through the stage of univalent division like the rest. No cases of restitution nuclei following division I have been observed directly, and chromosomes are rarely left out of the two interphase nuclei. In 135 pollen mother cells at interphase, only two were seen to have any chromosomes omitted from the two regular nuclei.

Each of the interphase nuclei usually has fourteen single chromosomes (Fig. 17). The chromosomes usually show some supercoils, and certain of them have small nucleoli attached. In the pollen mother cell figured (Fig. 17), four of the chromosomes in the lower nucleus, and three of those in the upper nucleus, are seen to have

nucleoli. In general, four chromosomes of the diploid complement elaborate small nucleoli at interphase, two of them nearly terminally and two in a more proximal position.



Figs. 17-18. *Oenothera decipiens*. Pollen mother cells at interphase and metaphase II respectively; they follow on an anaphase I division of fourteen univalents.

Where there have been one or more bivalents at metaphase I, each of the interphase nuclei has one or more of the typical double-arc double chromosomes, and correspondingly fewer univalents. At the second division which follows there are regularly two spindles. Thus, of 134 pollen mother cells at division II only one had a single spindle with about twenty-eight chromosomes; a second one had two chromosomes in the cytoplasm left out of the two main spindles. Accurate counts of the numbers of single and double chromosomes on both of the spindles at metaphase II are difficult to make, chiefly owing to the small chromosome size and to the overcrowding of the spindles. Table II summarizes what data could be obtained;

Table II. *Numbers of single and double chromosomes on the second division spindles*

First spindle	Second spindle	Numbers of pollen mother cells observed
14 single chromosomes	14 single chromosomes	23
1 double and 13 single chromosomes	13 single chromosomes	1
2 double and 12 single chromosomes	12 single chromosomes	1
1 double and 12 single chromosomes	1 double and 12 single chromosomes	1
2 double and 10 single chromosomes	2 double and 10 single chromosomes	1
28 single chromosomes	—	1
	Total...	28

the frequencies do not accord very well with those of their prototypes at metaphase I. The class with 14 chromosomes on each spindle is very large compared with the frequency of 14 univalents at metaphase I. But, as Fig. 18 shows, the chromosomes

are widely scattered, usually from pole to pole, at division II. Therefore it is difficult to determine whether some of the spindles have had a double chromosome which has already divided. The second and third records in Table II show that univalents occasionally pass undivided to one or other pole at anaphase I.

The second division differs conspicuously from the first in that there is no congression of the chromosomes at the equator of the spindle. After a time, the univalents go towards one or other pole or remain about midway between the two poles. As a result of this, the tetrads are made up of various numbers of cells, usually from two to four. The proportions of these found in a small count were:

No. of cells in "tetrad"	2	3	4	5
No. of "tetrads"	29	18	87	7

Diads result from a restitution nucleus following each division II spindle; triads from a restitution following one of the two spindles; and tetrads from non-occurrence of restitution nuclei after either spindle. In addition, there are sometimes one or more supernumerary nuclei, developed from small groups of chromosomes, one or more in number, that have been omitted from the main groups.

The functional pollen grains should be mainly diploid, since the approximately haploid pollen grains resulting from the triads and tetrads would rarely contain a set of all the haploid chromosomes. A number of functional grains would also have one or more of the chromosomes in duplicate. The samples of "tetrads" counted should yield 495 potential pollen grains, of which $(2 \times 29) + 18 = 76$ (about 15 %) should be approximately diploid. No counts of good grains amongst the mature pollen are available, though a casual examination has shown them to be very few relative to the larger number of empty shrunken grains of various sizes. The following counts of the numbers of grains with various germ-pore-lobe numbers were derived from young pollen grains before the good had become differentiated from the bad.

No. of germ-pore-lobes per pollen grain	1	2	3	4	5	6
No. of pollen grains	13	109	139	142	30	3

Possibly the estimated number of those with a few lobes may be too low, as they are the smaller grains more readily squeezed out around the edge of the preparation. Grains with four or more lobes (40 % of the total) will include those that are diploid, as well as those that are disomic for one or more chromosomes.

Genetical behaviour

The two complexes, as de Vries' (1919) genetical studies show, are respectively *velans*, presumably unchanged from its *Lamarckiana* constitution, and *decipiens* (*haplo decipiens*). The latter is a mixture of *velans* and *gaudens* chromosomes and it is viable in homozygous condition as the *decipiens* individuals.

Since *decipiens* is a homozygous segregate it would be expected to have seven pairs of chromosomes at meiosis. The only cytological observations, apart from the failure of pairing, at all incompatible with this expectation are the two chain tri-

valents observed in two pollen mother cells out of over fifteen hundred. These are probably exceptional and are perhaps similar in causation to trivalents in haploid *Oenothera* (Catcheside, 1932), where indeed they are more frequent.

The cytological evidence shows that the two complexes of *O. erythrina* differ by interchanges involving three chromosomes. The chromosomes of ^h*decipiens* may be identified in terms of standard interchanges by means of suitable crosses. The relevant data, obtained from progenies grown at Pasadena, is in Table III.

Table III

Cross	Hybrid combinations	Chromosome configurations
<i>erythrina</i> × <i>blandina</i>	<i>e-velans</i> . ^h <i>blandina</i>	8, 2, 2, 2
<i>erythrina</i> × <i>rubricalyx-α</i> (hom.)	^h <i>decipiens</i> . ^h <i>blandina</i>	4, 2, 2, 2, 2, 2
	<i>e-velans</i> . <i>rubricalyx-α</i>	6, 2, 2, 2
	^h <i>decipiens</i> . <i>rubricalyx-α</i>	2, 2, 2, 2, 2, 2, 2
<i>erythrina</i> × <i>Hookeri</i>	<i>e-velans</i> . ^h <i>Hookeri</i>	4, 2, 2, 2, 2, 2
	^h <i>decipiens</i> . ^h <i>Hookeri</i>	4, 2, 2, 2, 2, 2
<i>erythrina</i> × <i>purpurata</i>	<i>e-velans</i> . ^h <i>purpurata</i>	6, 2, 2, 2, 2
	^h <i>decipiens</i> . ^h <i>purpurata</i>	6, 2, 2, 2, 2
<i>erythrina</i> × <i>suaveolens</i>	<i>e-velans</i> . <i>flavens</i>	4, 4, 2, 2, 2
	^h <i>decipiens</i> . <i>flavens</i>	4, 4, 2, 2, 2
<i>erythrina</i> × <i>Lamarckiana</i>	<i>e-velans</i> . <i>gaudens</i>	12, 2
	^h <i>decipiens</i> . <i>gaudens</i>	10, 2, 2
	^h <i>decipiens</i> . <i>velans</i> (<i>erythrina</i>)	6, 2, 2, 2, 2

The known chromosomal constitutions of the interchange complexes, in terms of the end pairing segments of the chromosomes, are listed below. The definitions of the ends are those proposed by Emerson & Sturtevant (1931), with the exception of the emendation suggested by Catcheside (1937). Some of the formulae are based on partly unpublished data:

^h <i>Hookeri</i>	1·2	3·4	5·6	7·8	9·10	11·12	13·14
<i>flavens</i>	1·4	2·3	5·6	7·8	9·10	11·12	13·14
<i>velans</i>	1·2	3·4	5·8	6·7	9·10	11·12	13·14
<i>gaudens</i>	1·2	5·6					
^h <i>blandina</i>	1·2	3·4	5·6	7·10	11·12	8·13	9·14
<i>rubricalyx-α</i>	1·2	3·4	5·6	7·14	8·13	9·10	11·12
^h <i>purpurata</i>	1·2	3·4	5·6	7·10	8·90	11·12	13·14

Since ^h*decipiens* gives seven pairs of chromosomes with *rubricalyx-α* at meiosis, it follows that the arrangement of the ends of the chromosomes is identical in the two complexes: ^h*decipiens* is 1·2 3·4 5·6 7·14 8·13 9·10 11·12. This conclusion is compatible with all the test configurations given with other complexes. *Erythrina-velans* gives one ring of four chromosomes with ^h*Hookeri* and two with *flavens*. It therefore has two ^h*Hookeri*, not *flavens* chromosomes, namely 1·2 and 3·4. The chromosome in common with *gaudens* is accounted for by 1·2; therefore *erythrina-velans* does not have 5·6. It gives a ring of six chromosomes with ^h*purpurata* and a ring of four with ^h*Hookeri*; *erythrina-velans* must have one of the two ^h*Hookeri* not ^h*purpurata* chromosomes, that is 7·8 or 9·10. Further, on similar grounds, *erythrina-velans* must have one of the two ^h*Hookeri* not *rubricalyx-α* chromosomes, that is 7·8

or 13·14. Putting these facts together, we can see that *erythrina-velans* has 7·8 but not 5·6, 9·10 or 13·14, or else 9·10 and 13·14 but not 5·6 or 7·8. The first possibility must be ruled out since there could be only four chromosomes in common between *erythrina-velans* and ^h*Hookeri*. Hence *erythrina-velans* has 9·10, 13·14 and also 11·12 to make the fifth pair with ^h*Hookeri*; it also has an interchange between 5·6 and 7·8.

$$\text{Erythrina-velans is } 1\cdot2 \quad 3\cdot4 \quad 9\cdot10 \quad 11\cdot12 \quad 13\cdot14 \quad \begin{cases} 5\cdot8 & 6\cdot7 \text{ (a)} \\ 5\cdot7 & 6\cdot8 \text{ (b)} \end{cases}$$

The first alternative (a) is identical with the formula of standard *velans* and there is no reason for supposing that *erythrina-velans* differs from the normal. But we cannot decide between the alternatives on our present information. It happens that this limitation does not influence the identification of the chromosome carrying the asynaptic gene.

If the formulae of ^h*decipiens* and *erythrina-velans* are compared, it will be seen that the four bivalents at meiosis in *O. erythrina* are accounted for by the chromosomes 1·2, 3·4, 9·10 and 11·12. The ring of six chromosomes is made up of 5·6, 7·14 and 8·13 from ^h*decipiens* and 5·8, 6·7, and 13·14 or 5·7, 6·8 and 13·14 from *erythrina-velans*.

If the asynaptic condition is controlled by a single recessive gene it should show simple Mendelian segregation in the progeny of a hybrid in which it was in a bivalent. This must be the case in *rubricalyx-α*.^h*decipiens* which has seven bivalents. In this and other segregations, classification for asynapsis was made on the evidence of male fertility or sterility. Asynaptic individuals have thin, rather twisted, indehiscent anthers. An *F*₂ family grown from this hybrid showed 43 normal to 11 asynaptic, which is a reasonable agreement with a 3 : 1 ratio (*P* = 0·62). The same family also shows that the asynaptic gene is independent of the *P* locus. *Rubricalyx-α* has the fully red *rubricalyx* buds (*P*^r) which are dominant to the striped buds (*P*^s) of *decipiens*. The family gave 30 *P*^r and 13 *P*^s non-asynaptics and 11 *P*^r and 0 *P*^s asynaptics. We may now designate the asynaptic gene by the symbol *as*.

The various *erythrina* families grown have given the following results:

	<i>erythrina</i>		<i>decipiens</i>	
	+	<i>as</i>	+	<i>as</i>
1936	14	0	0	19
1937	15	0	0	15
1938	85	2	0	23
Totals	114	2	0	57

These show that *O. erythrina* is heterozygous for the gene *as* which is coupled to the ^h*decipiens* complex with about 1 % of crossing-over to the *velans* complex. This means that the locus of the gene is in one of the three ^h*decipiens* chromosomes, 5·6, 7·14 and 8·13, which are in the ring of six chromosomes formed at meiosis in

erythrina, and which together carry the genes by which *^hdecipiens* differs from *velans*. The chromosome concerned may be found by testing each one in crosses in which the chromosomes are marked genetically.

In *flavens* chromosome 5·6 has been shown by Renner (1933) to carry *Sp* (pointed buds and leaves). It is lethal in homozygous condition. Hence, if *as* is in chromosome 5·6 of *^hdecipiens*, it should show linkage in coupling phase with *sp*, the allele of *Sp* carried by *^hdecipiens*. Two small families together gave six *P^s As Sp flavens. ^hdecipiens* and one *p as sp lutescens (flavens. flavens)*. The *lutescens* individual had defective flowers (reduced number of petals, etc.); the others had normal flowers. The evidence is too slender to be of value, but it is compatible with other evidence that *as* is in chromosome 5·6.

In *gaudens. ^hdecipiens* the two pairs are accounted for by chromosomes 1·2 and 5·6; chromosomes 7·14 and 8·13 of *^hdecipiens* are in the ring of ten chromosomes. If the gene *as* is 5·6 it should segregate independently of the complexes *gaudens* and *^hdecipiens* determined by the chromosomes in the ring of ten. *Gaudens* carries *p* for green buds and non-punctate stems, while *^hdecipiens* carries *P^s* for red striped buds and punctate stems; the loci of these genes are in chromosome arm 3, which is in the ring of ten chromosomes in this combination. The combination *P^sp* is readily separable from *P^sP^s*. Three *F₂* families from *gaudens. ^hdecipiens* gave the following results:

Family	<i>P^sP^s ^hdecipiens</i>		<i>P^sp gaudens. ^hdecipiens</i>	
	+	<i>as</i>	+	<i>as</i>
102	14	11	15	0
102 A	67	19	8	2
138	41	13	18	4
Totals	122	43	41	6

There is a considerable, though not significant ($P=0.05$), deficiency of asynaptic *gaudens. ^hdecipiens*. In any case, the ratios are distorted by *gaudens* and *^hdecipiens* competition. They clearly show that *as* is in chromosome 5·6, since the character segregates independently of the complex differences and of the *P* locus which is situated in the ring of ten chromosomes.

Other *F₂* segregations in the *F₁* of which chromosome 5·6 was a bivalent showed reasonable Mendelian ratios. No inference concerning the chromosome bearing *as* can be drawn from them, for no known chromosomes were marked by genes giving visible characters and no cytological examinations were made to discover the interchange heterozygotes. Thus *^hblandina. ^hdecipiens* gave 79 *As* and 20 *as* ($P=0.27$); of these 9 *As* and 6 *as* were classified as *^hdecipiens. ^hdecipiens* plants, but the separation was by no means clear. The combination *^hpurpurata. ^hdecipiens* gave 8 *F₂* plants all of which were *purpurata*; 5 were *As* and 3 *as*.

Finally, *^hHookeri. ^hdecipiens* gave an *F₂* of 67 *As* and 12 *as* plants ($P=0.04$); 10 *As* and 5 *as* were pale green plants, the remainder being fully green. *O. erythrina* has chloroplasts which are genetically identical with those in *Lamarckiana*. The complex

^h*Hookeri* in homozygous condition interferes with chlorophyll development in *Lamarckiana* and *erythrina* plastids, giving white or pale green seedlings which become greener with age unless they die first (Renner, 1924). *Velans* has an effect identical with that of ^h*Hookeri*, so ^h*Hookeri.velans* plants are pale green when they carry *Lamarckiana* plastids. ^h*decipiens* permits normal chlorophyll development in *Lamarckiana* plastids. This effect is genetically dominant to those of *velans* and ^h*Hookeri*. Hence *erythrina (velans. hdecipiens)* and ^h*decipiens. hHookeri* plants from the cross *erythrina* × *Hookeri* are fully green. All *decipiens* segregates from *erythrina* are fully green. Therefore the gene or genes permitting normal chlorophyll development is linked to the gene for asynapsis and is situated somewhere in chromosomes 5·6, 7·14, and 8·13. That giving the defective condition must be in the corresponding chromosomes of *velans*. About a quarter (27 out of 102) of the *F*₂ seedlings from ^h*Hookeri. hdecipiens* (from *erythrina* × *Hookeri*) were pale; many died in the seedling stage so that only 15 were scored for male sterility. The defective chlorophyll development cannot be due to any genes carried by chromosome 5·6, for no repulsion linkage with the gene for asynapsis has been displayed. Whether a single gene or a complex of genes is concerned cannot be said until chromosomes 7·8 and 13·14 of ^h*Hookeri* have been tested separately in a suitable cross.

SUMMARY

In a particular strain of *Oenothera erythrina*, the alethal complex ^h*decipiens* carries a recessive gene (*as*) for asynapsis in chromosome 5·6. In homozygous condition it causes failure of bivalent formation at meiosis in the pollen mother cells, and probably also in embryo sac mother cells. Such plants are highly male and female sterile.

The complex ^h*decipiens* has the chromosomes 1·2, 3·4, 5·6, 7·14, 8·13, 9·10, 11·12.

In *O. erythrina*, chromosome 5·6 is in the ring of six chromosomes which includes the lethal complex of *velans*. The gene *as* is coupled with ^h*decipiens* with about 1 % of crossing over to *velans*.

In *gaudens. hdecipiens*, chromosomes 1·2 and 5·6 form pairs and 7·14 and 8·13 are in the ring of ten chromosomes; *as* segregates independently of the complexes and is therefore in chromosome 5·6.

In ^h*decipiens. hHookeri* chromosome 5·6 forms a pair and 7·14 + 8·13 of ^h*decipiens* forms a ring of four chromosomes with 7·8 + 13·14 of ^h*Hookeri*. The gene *as* segregates independently of the ^h*Hookeri* gene (or genes) impairing chlorophyll development in *Lamarckiana* plastids. It is probable that the genetic basis for this chlorophyll defect is in chromosomes 7·8 and 13·14 of ^h*Hookeri*, since a genetically similar one in *velans* does not segregate from *velans. hdecipiens* and therefore must be in 5·8, 6·7 and 13·14.

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A NEW SAPROPHYTIC GENUS OF THE VOLVOCALES

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(With 2 figures in the text)

INTRODUCTION

DURING the autumn of 1938 a peculiar alga was found in the grounds of Reading University. It appears to be a new genus of the interesting saprophytic section of the Volvocales.

DESCRIPTION

This alga occurs in various forms which can be arranged in a series and which are regarded as the stages in the development. It will be convenient to regard the youngest stage, i.e. the daughter cell as it emerges, as typical, and this will be described first, then the mature form, and then cell division to form new daughter cells.

The youngest stage of the organism (Fig. 1A) is a small active cell which swims with a rapid rotation. There is a central homogeneous body which appears to be a nucleus or nucleolus surrounded by a clear space. A little behind the anterior end is a small orange eyespot which is rather longer than broad and is most easily seen by mounting in dilute acetic acid. It appears to be a flat plate in the surface layer of protoplasm but occasionally appears round and deeper in colour owing to being strongly curved (C-shaped in end view). Both the eyespot and nucleus are difficult to see in living cells loaded with starch. No pyrenoid or contractile vacuoles were observed. The cell wall is thin and the protoplasm is in contact with it but it is readily separated by plasmolysis in strong glycerine. Small, round starch grains are normally abundant and are scattered through the protoplasm particularly in the middle region. On starvation it disappears first from the anterior and lastly from the posterior end.

As it matures the cell as a whole increases in size, but the cell wall extends more rapidly than the protoplasm so that it grows away from the protoplasm. At the same time as this process of inflation of the wall occurs, the arms diminish in size. Thus a considerable variety of intermediate forms are found of which Fig. 1C is a typical example. The wall gradually assumes the form of a thick spindle which is squarish in section, the corners of the square representing the four arms in their last stages of development. The change is not due to mere stretching as the wall now appears to be thicker than before. The protoplasm is most commonly spindle-shaped in lateral view, and square or circular in end view, the lateral and often the posterior projec-

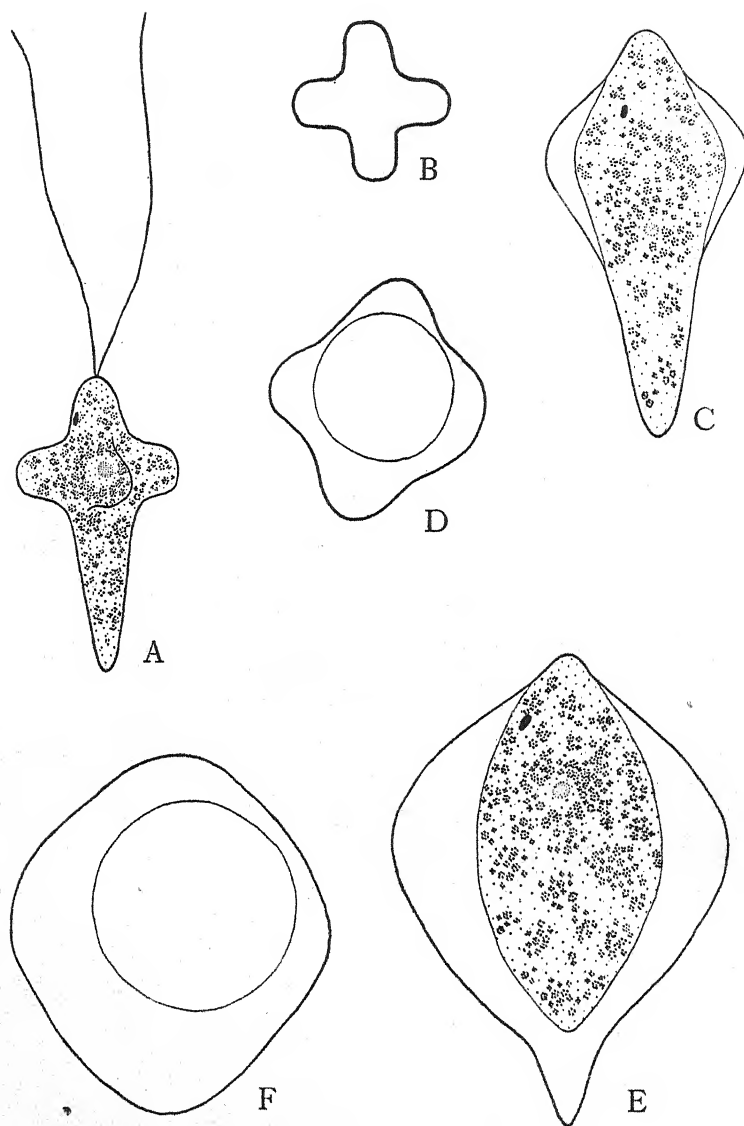


Fig. 1. Stage in development of *Hyalobrachion omphalotus*. A, shortly after liberation, showing nucleus, eyespot and starch grains. B, end view of A. C, intermediate stage (flagella omitted). D, end view of C showing protoplasm and wall becoming inflated. E, mature stage (flagella omitted). F, end view of E. $\times 2000$.

tions being withdrawn. At this stage (Fig. 1E) the cell is much larger and the organism swims much more slowly. The gap between the protoplasm and the wall is occupied by a clear fluid. On lightly staining in methylene blue, blue particles eventually precipitate in the cavity and their brownian motion proves the fluid nature of the medium. The nature of the wall is doubtful. It stains strongly in methylene blue and gentian violet but is fairly resistant to strong acids and alkalis. No positive cellulose reaction with iodine in zinc chloride or iodine and sulphuric acid could be obtained, but in view of its thinness this negative reaction may not be conclusive.

Cell division occurs in the motile condition, the mother cell remaining motile up to the time of liberation of the daughters. The protoplasm divides into two and then four oval masses which become elongated and develop the lateral "arms". The daughter cells become motile at an early stage in their development and shortly before liberation they swim actively inside the mother cell wall. Liberation occurs by the bursting of the parent wall, and the process is induced to occur slightly prematurely under the stimulus of very weak iodine solution.

All the stages show a response to the stimulus of light.

Under the abnormal conditions of a hanging drop culture the daughter cells are often liberated at the spindle shaped stage in their development, before the "arms" have been formed. Under these conditions also, strange branched specimens were found.

Cell division, the formation of daughter cells and their liberation, took place in about an hour and a half in an individual observed continuously in a freshly made hanging drop. The process took much longer in an older hanging drop.

In old hanging drop cultures many individuals appear to die and dissolve but others pass into a resting condition in which the protoplasm becomes spherical and surrounds itself with a mucilaginous wall. Cell division may occur in this state. No resting cysts with resistant walls were observed.

COMPARISON

Hyalobrachion belongs to the group of saprophytic Volvocalean genera which closely resemble a green form in shape but lack the plastid (Pascher, 1927).

Hyalobrachion resembles *Polytoma* from which however it is distinguished by its four lateral "arms", and even at its most inflated stage by its square shape in section. It closely resembles *Brachiomonas* in shape but is distinguished by its lack of chlorophyll.

What must be a second species of this genus was observed in this district by Prof. T. M. Harris. He recognized only the four "armed" form which differed in having much more slender and pointed "arms" which were directed slightly backwards. Here also, signs of inflation of the wall were noted.

MATERIAL AND TECHNIQUE

Hyalobrachion omphalotus was found during the autumn and winter of 1938-9 in a stagnant ditch containing quantities of fallen leaves. It had been noticed in the

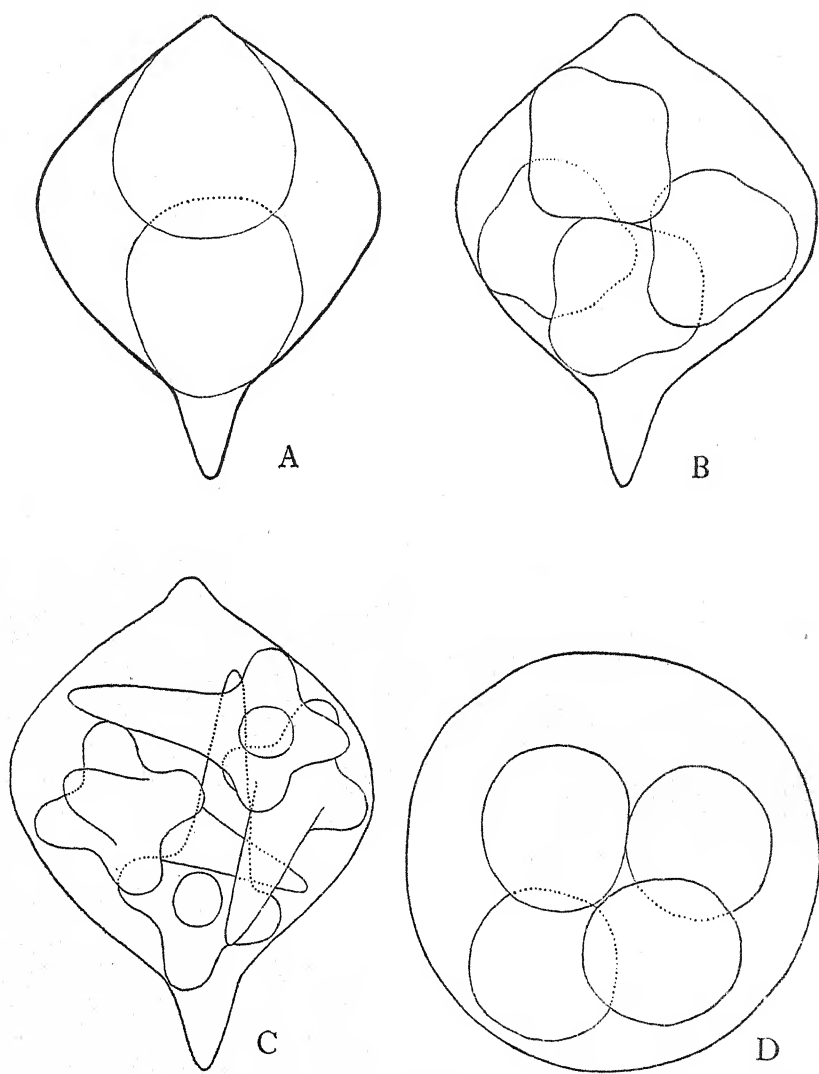


Fig. 2. A, B and C, stages in reproduction of *Hyalobrachion omphalotus*. A, protoplasm divided into two (flagella omitted). B, protoplasm divided into four, lateral projections beginning to develop (flagella omitted). C, parent cell just before liberation of daughters (flagella omitted). D, non-motile form developed in culture. $\times 2000$.

same ditch in 1937-8 by Prof. Harris who states that it appeared in the autumn and flourished through the winter but disappeared in spring, being replaced by other saprophytic flagellates. The water, though rich in organic matter, was not at all foul

smelling and had a considerable flagellate flora including a number of other saprophytes.

The other species noted by Prof. Harris was found in winter in a shallow pond containing cow-dung and leaves, a locality where it must have endured many months' desiccation.

Hyalobrachion can be readily concentrated by centrifuging, although the process seems to cause the death of a considerable proportion of the specimens.

The only special methods used in observation were to make hanging drop cultures in which it usually survived for four days but on one occasion for a fortnight. A method of immobilizing the active cells was to mix the mounting fluid with a little recently cooled but unsolidified gelatine which soon sets without harming the organisms. In such a mount it was possible by moving the coverslip, to obtain an end view which can rarely be seen in a water mount.

DIAGNOSIS

Hyalobrachion gen.nov.

Structura cellulae unicae pariete definito et duobus flagellis aequalibus. Chloroplastum absens, et protoplasma pellucidum. Stigma praesens. Subsidium alimenti ex amylo constat. Cellula producta, quattuor prominentias ferens in modum radorum positas, brachiorum similes, hae a lateribus ortae. Alimentum saprophyticum. Regeneratio per conformationem cellularum quattuor, vel nonnunquam plurium, ex cellula matris natarum. Regeneratio per sexum non cognita.

H. omphalotus sp.nov.

Hyalobrachion cuius cellula primum 18μ longa, 10μ lata. Quattuor prominentias obtusas ferens, vix ante mediam partem positas, ad latera inclinatas. Extremum superius obtusum, posterius acutum. Cellulae seniores maiores fiunt (30μ longae, 24μ latae), paries inflata humore fit, specie fusi lati similis ab lateribus visa, quadrata paene tamens ab extremo visa. Protoplasma aut specie fusi similie aut globosum. Paries tenuis. Stigma rubellulum, 4μ ab extremo superiore positum. Micae parvae amyli per protoplasma sparsae. Pyrenoidum absens. Flagella parvo longiora corpore.

In conclusion I wish to thank Prof. T. M. Harris for his kindly criticism and help in the preparation of this paper.

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PHOSPHORYLATION AND RESPIRATION IN BARLEY

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(With 5 figures in the text)

THE occurrence of various compounds of phosphorus in young barley has been examined in a previous paper. Their relation with the seedlings' respiration has now to be considered.

METHODS

Phosphate determinations were carried out by the methods already described (Arney, 1939). Inorganic, esterified and total phosphate were the categories directly determined. The residual phosphate [= total phosphate - (inorganic + esterified)] is mainly composed of phospholipoids and phosphoproteins, and has not been considered in detail. Inorganic and esterified phosphate are determined in one and the same extract; but total phosphate requires a separate sample. Residual phosphate has, therefore, to be determined as between independent samples, and is accordingly liable to an increased error. Pairs of "total phosphate" determinations are averaged for the calculation (Tables I, II, IV). In the analyses of "seedlings" only the growing tissues were taken; the residue of the endosperm was always discarded.

Respiration. The output of carbon dioxide was measured by the Pettenkofer method, with an automatic switch-over from one absorption tube to the next. The apparatus was duplicated so that two experiments could be run together. The grains were germinated on sand in respiration chambers exactly as described in the previous paper. A wider range of phosphate concentration was employed, viz. 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5 and 2.0. Unit phosphate implies that of a complete culture solution, viz. 0.25 g. $\text{CaH}_4(\text{PO}_4)_2\text{H}_2\text{O}$ per litre. Substitution was usually by means of calcium nitrate, but in one experiment calcium chloride was used without its making any difference. In most experiments the culture solution was supplied at the start and not afterwards altered. A chamber was modified to allow of further doses being introduced without opening up or other disturbance. Small quantities of culture solution were then introduced daily. Numerous determinations were made of the respiration in this and a normal chamber, but the mean respiration rates in the two chambers did not differ significantly.

When first made up, the culture solution with full (1.0) phosphate had a reaction of pH 5.8, and the 0.0 phosphate solution a reaction of 6.0. After supporting 10 days' growth they had changed to 6.7 and 6.9 respectively. These two drifts, being close and parallel, could not introduce effects liable to be confounded with those due to differences of phosphate concentration.

During the first few days of germination, the rate of respiration increased very greatly. The experiment was, therefore, made of increasing the movement of air through the germination chambers. This did not result in any change of the respiration rates and it may, therefore, be assumed that anaerobiosis and carbon dioxide narcosis do not restrict them.

Table I. Grain of 1934 germinated and analysed about 7 months after harvest

Phosphate in culture medium	Age in days	CO ₂ output over last 12 hr. c.c. per 80 seedlings	mg. phosphate per seedling			
			Inorganic phosphate	Esterified phosphate	Residual phosphate	Total phosphate
0	3	30.28	.0443	.0215	.0493	.1202
			.0418	.0191		.1051
		Mean	.0430	.0203		.1126
0	5	41.56	.0899	.0324	.0858	.2126
			.0867	.0307		.1986
0	5	55.36	.0784	.0369	.0732	.2266
			.1221	.0468		.2040
0	5	63.20	.0853	.0496	.0947	.2420
			.0898	.0557		.2277
		Mean	.0920	.0430		.2186
0	10	39.44	.0774	.0298	.1490	.2440
			.0762	.0306		.2680
		Mean	.0768	.0302		.2560
0.2	5	45.60	.1044	.0338	.0832	.2135
			.0997	.0371		.2279
0.2	5	50.00	.0917	.0353	.0927	.2300
			.0929	.0382		.2135
		Mean	.0972	.0361		.2212
0.2	10	43.52	—	.0298	—	.2783
			.1144	—		—
0.2	10	38.00	.1549	.0457	.0682	.2849
			.1449	.0457		.2426
		Mean	.1374	.0404		.2686
0.4	10	—	.1759	.0504	.1439	.3232
			.1427	.0546		.3883
		Mean	.1593	.0525		.3557
0.6	10	43.20	.1870	.0575	.0769	.3018
			.1951	.0359		.3275
		Mean	.1910	.0467		.3146
1.0	3	32.34	.0529	.0208	.0584	.1358
			.0512	.0262		.1320
		Mean	.0521	.0235		.1339
1.0	5	51.60	.1237	.0386	.0900	.2492
			.1203	.0462		.2596
1.0	5	42.96	.1021	.0331	.0625	.1829
			.0976	.0345		.2093
1.0	5	44.40	.1053	.0374	.0684	.1959
			.0939	.0380		.2174
		Mean	.1071	.0379		.2190
1.0	10	42.40	.1904	.0416	.1062	.3489
			.1929	.0644		.3527
		Mean	.1916	.0530		.3508

Table II. *Grain of 1936 harvest*

Phosphate in culture medium	Age in days	CO ₂ output over last 24 hr. in c.c. per 80 seedlings	mg. phosphate per seedling			
			Inorganic phosphate	Total esters	Residual phosphate	Total phosphate
o	5	139	·0701	·0336	·0406	·1443
o	5	126	·0677	·0356	·0401	·1441
			·0622	·0416		·1431
o	5	136	·0808	·0335	·0390	·1517
			·0809	·0303		—
o	5	137	·0703	·0372	·0519	·1544
			·0673	·0337		·1578
o	5	122	·0676	·0348	·0511	·1480
			·0695	·0314		·1574
		Mean	·0707	·0347		·1509
o	7	124	·1035	·0262	·0647	·1945
			·1009	·0281		·1936
o	7	126	·0998	·0368	·0504	·1880
			·1016	·0370		—
		Mean	·1015	·0320		·1920
1·0	5	151	·1023	·0373	·0228	·1560
			·1026	·0382		·1700
1·0	5	131	·0835	·0524	·0455	·1790
			·0852	·0466		·1797
1·0	5	125	·1064	·0374	·0425	·1912
			·1056	·0371		·1802
1·0	5	137	·0921	·0349	·0459	·1727
			·0940	·0293		·1694
1·0	5	—	·1188	·0359	·0282	·1829
1·0	5	—	·1109	·0268	·0300	·1677
		Mean	·1001	·0376		·1749
1·0	7	127	·1414	·0386	·0353	·2048
			·1355	—		·2259
1·0	7	139	·1450	·0150	—	—
			·1546	—		—
1·0	7	—	·1312	·0318	·0495	·2250
			·1359	·0259		·1987
1·0	7	—	·1361	·0263	—	—
		Mean	·1400	·0275		·2136

At the close of each experiment the chambers were opened and examined for fungal and bacterial contaminations. Occasionally fungal mycelia were found and the experiment was discarded. After several of the experiments the sand in which the seedlings had been growing was washed with a little sterile distilled water and the washing examined under the microscope. The field of the 1/6 in. objective never showed more than four bacteria at a time.

The carbon-dioxide output with varying phosphate supply. Numerous experiments were performed in which grains of the 1932, 1933, 1934 and 1936 harvests were germinated with culture solutions of varying phosphate content. Their results are sufficiently summarized by Fig. 1, A-D, since in no case did the full range of phosphate supply produce a differentiation of respiration rates. The reason for this

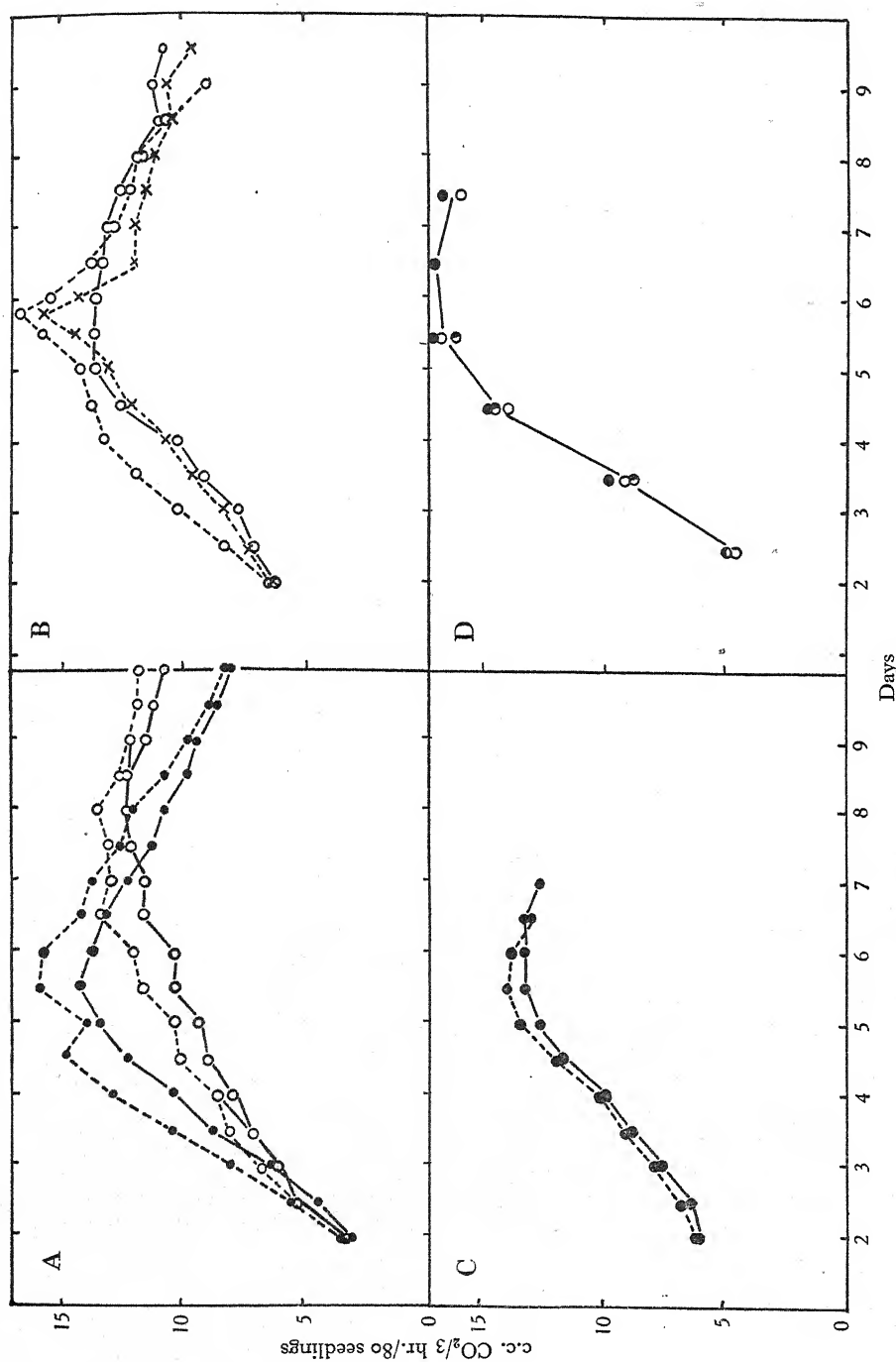


Fig. 1. Carbon-dioxide output of seedlings from whole grains with varying doses of phosphate and from different harvests.

- A, ●—● 1932 harvest; full phosphate: ●—● 1932 harvest; no phosphate: ○—○ 1933 harvest; no phosphate.
 B, 1934 harvest; ●—● full phosphate: ×—× 0.2 phosphate: ○—○ no phosphate. Experiments in March and April 1935.
 C, 1934 harvest; ●—● full phosphate: ●—● full phosphate: ●—● no phosphate. Experiments in May 1935.
 D, ● 1936 harvest; full phosphate: ○ 1936 harvest; no phosphate: ○ 1936 harvest; no phosphate.

is made clear by the analyses already recorded (Arney, 1939) and by the further results of Tables I and II. The seedlings raised on culture solutions devoid of phosphate contain a large amount of inorganic phosphate within themselves, which may be about half the total phosphate content. It is significant (Tables I and II) that, under these conditions, the uptake of additional phosphate from the solutions containing it, does not necessarily tend to increased formation of esters (cf. Arney, 1939).

The source of the excess phosphate in the young seedlings is in the endosperm. Advantage was, therefore, taken of the fact (first pointed out by Brown & Morris, 1890) that barley embryos may be separated from their reserve tissues without

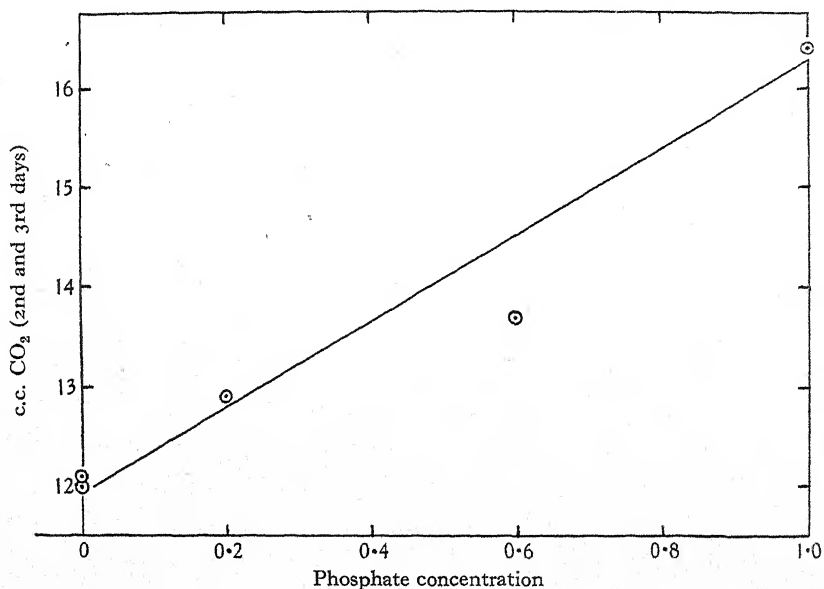


Fig. 2. Carbon-dioxide output by 120 excised embryos during the second and third days of germination, with varying phosphate concentration. Grain of 1933 harvest germinated in December.

injury and then germinated on artificial media. After the grain had been stripped of glumes and paleae and repeatedly washed in autoclaved water, the embryos were cut out and washed with further changes of sterile water and planted on the prepared sand enclosed in respiration chambers exactly as in experiments with whole grain.

Experiments were performed in this fashion with four different phosphate concentrations, and the carbon dioxide measured during the second and third days of germination. There was a steady increase of carbon-dioxide output with increasing concentration of phosphate (Fig. 2, and Table III, top line). Grain of the 1933 harvest was used, and the experiments were carried out in December of the same year. Attempts to repeat them in the succeeding months had unexpected results. The carbon-dioxide output with the lower phosphate concentrations showed a progressive increase with time which had little counterpart at the upper end of the

phosphate scale (Table III) and, by the end of February, the original relation was reversed.

Table III. *c.c. carbon dioxide emitted by 120 excised embryos during the second and third days of germination; planted on sand moistened with mineral culture solution only*

	Phosphate concentration							
	0	0.2	0.4	0.6	0.8	1.0	1.5	2.0
1-12 Dec. 1933	{ 12.1 12.0	12.9	—	13.7	—	16.4	—	—
3-19 Jan. 1934	—	—	16.2	—	18.2	—	—	—
22-27 Jan. 1934	18.9	—	18.0	18.3	—	17.4	20.1	19.6
12-28 Feb. 1934	{ 21.8 22.7	—	—	—	—	18.4 19.7	—	—

Later experiments with 1934 grain again showed a small and rather doubtful increase of CO₂ output with rising phosphate concentration, viz.

PO ₄	0.0	0.2	1.0
CO ₂	{ 22.9 15.7	20.8 —	21.2 20.9
Mean	19.3	20.8	21.1

The greatest output of carbon dioxide obtained under these conditions is only about a tenth of the corresponding output from complete grains. After the first 24 hr. the isolated embryos suffer acute carbohydrate starvation (A. L. James, 1938) and this is no doubt a major cause of the respiration restriction. Addition of sucrose to the artificial medium leads to the expected increase of respiration rate with an optimum concentration at about 4 % (A. L. James, 1938).

The presence of sucrose in the medium increases the difficulty of keeping down moulds and bacteria in the respiration chambers, and some experiments became contaminated. These are excluded from the following results and only those experiments are quoted in which it seems impossible that anything other than the barley could have materially affected the carbon-dioxide output. Clean experiments were finally obtained for six different concentrations of phosphate. The individual time curves are given in Fig. 3, and the summed carbon dioxide from the end of the first to the end of the sixth day in Fig. 4. The first 24 hr. are rejected (as in previous experiments), since their anomalous results may be due to slow entry of the phosphate. It is clear from these experiments that, in the presence of an adequate carbohydrate supply, genuine phosphate starvation may restrict the respiration rate. There is a variation of 60 % over the range of phosphate concentration employed, and the linearity of the curve suggests that further increases might have been expected with stronger phosphate doses.

The relation between esterified phosphate and respiration rate. A number of estimations of esterified phosphate were carried out on seedlings from 1934 grain.

The respiration of the seedlings was also followed, and the CO_2 output for the last 12 hr. before harvesting is shown in Table I. The artificial control of phosphate in the culture medium did not have any definite effect either upon the respiration rate or on the content of esterified phosphate, and may be ignored. Considerable variations were noticeable none the less, due partly to the fact that the samples used

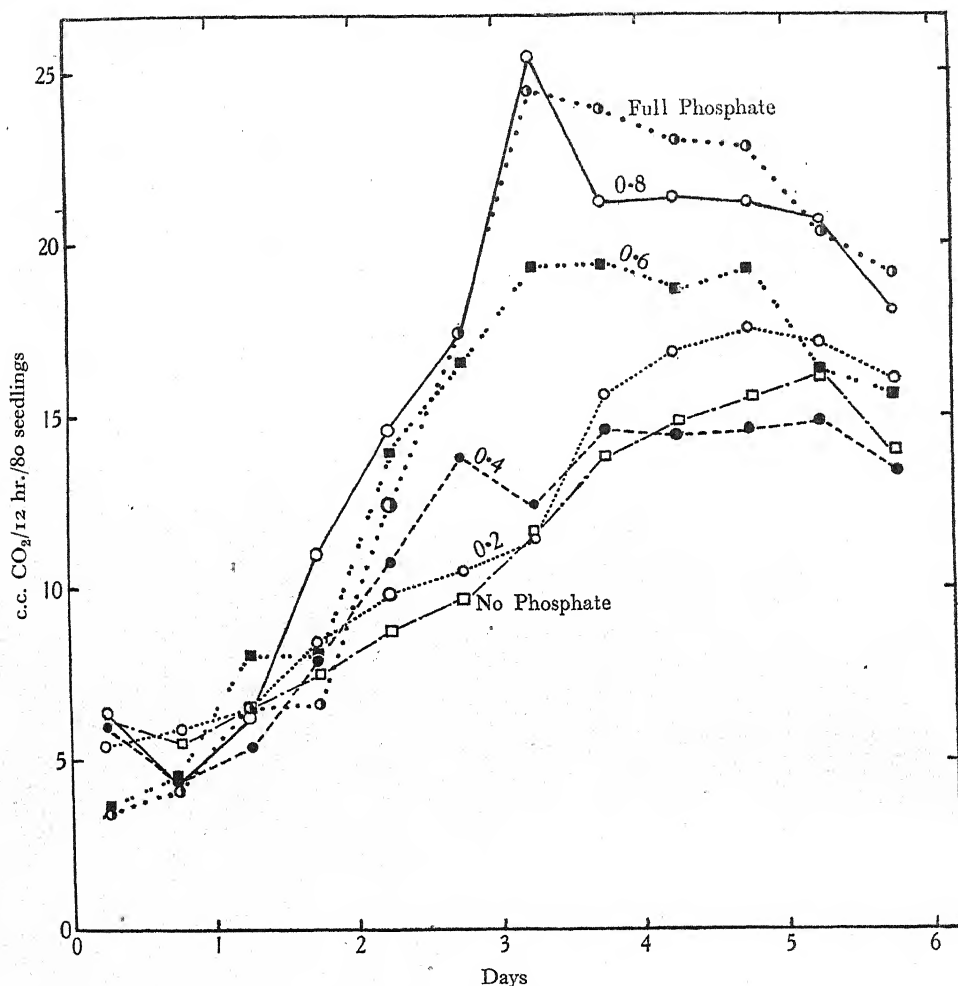


Fig. 3. Time curves of carbon-dioxide output by embryos germinated on a medium containing sucrose with various phosphate concentrations. The figures above the curves show the concentration of phosphate supplied.

were small. Pairs of analytical samples were derived from a single respiration experiment, and have been averaged for comparison. In Fig. 5 the respiration values and mean values of esterified phosphate from Table I are plotted against one another. Their correlation in 5-day-old seedlings is very obvious ($r=0.89$; $P<0.01$), and the 3-day-olds fall on the same regression line with lower coordinates. The

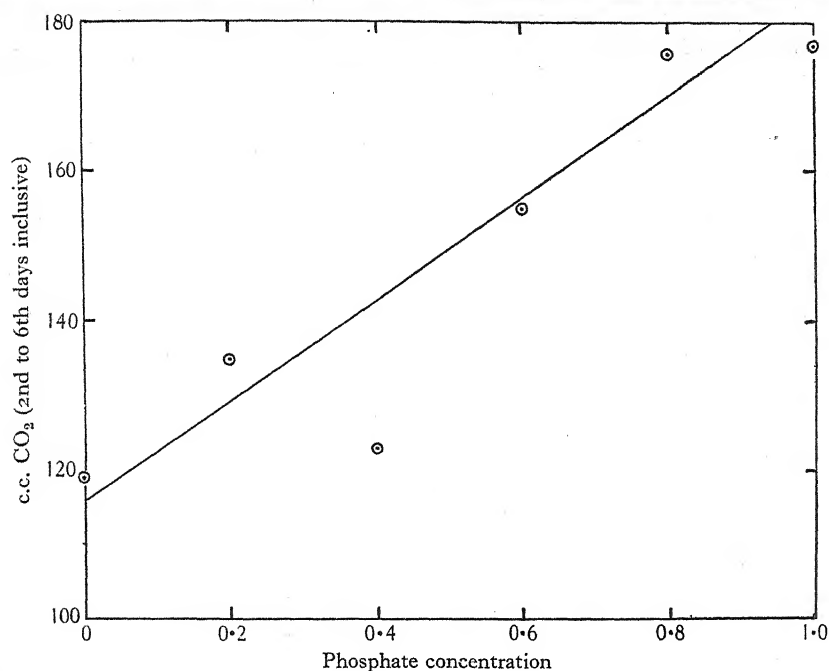


Fig. 4. Carbon-dioxide output by eighty embryos germinated on media containing 4% sucrose and various phosphate concentrations. The carbon dioxide emission is summed from the second to sixth days inclusive.

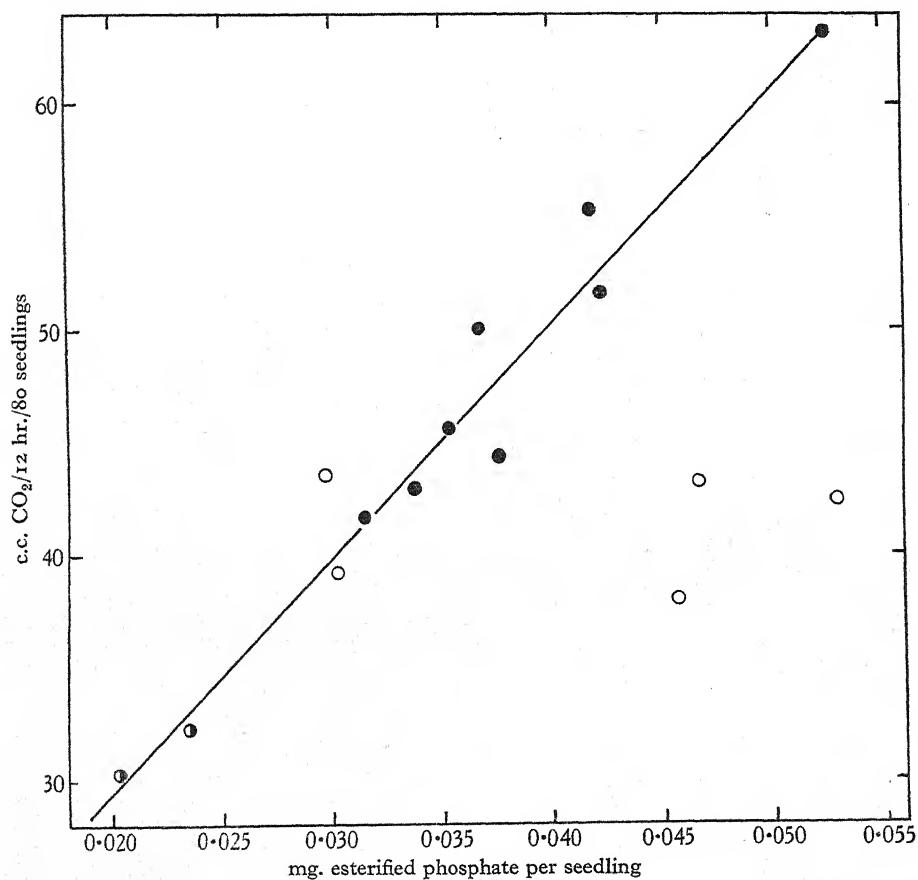


Fig. 5. Carbon-dioxide emission for 12 hr. prior to analysis of seedlings from entire grain (1934 harvest) plotted against content of esterified phosphate.

● 3-day seedlings; ● 5-day seedlings; ○ 10-day seedlings.

position after 10 days is strikingly different; the correlation has entirely disappeared, and the respiration rate is restricted to about 43 c.c. per 12 hr., irrespective of ester content. The last seedlings are subject to carbohydrate starvation (A. L. James, 1938).

In the experiments with 1936 grain, owing to improved sampling, the variation both of respiration and of esterified phosphate was much reduced (Table II), and the method of correlation is no longer useful.

Analyses were also made of embryos from 1934 grain germinated without their endosperms (Table IV). The samples were numerically larger, each containing

Table IV. *Embryos from 1934 grains germinated without endosperms*

Phosphate in culture medium	Age in days	CO ₂ output over last 12 hr. c.c. per 80 embryos	mg. phosphate per seedling			
			Inorganic phosphate	Esterified phosphate	Residual phosphate	Total phosphate
0	2	3.69	.0287 .0215	.0079 —	.0171	.0536 .0538
0	2	3.82	.0251 .0253	.0083 .0126	.0140	.0512 .0510
		Mean	.0252	.0096		.0524
0	4	3.45	.0255 .0260 .0268	.0044 .0033 .0040	.0145	.0466 .0413 .0455
0	4	2.87	.0272 .0312 —	.0057 .0075 .0063	.0136	.0467 .0503 .0522
		Mean	.0273	.0053		.0471
0.2	2	3.67	.0277 .0288 .0272	.0084 .0089 .0111	.0195	.0552 .0595 .0561
		Mean	.0279	.0095		.0569
0.2	4	2.77	.0301 .0308 .0321	.0084 .0068 .0072	.0151	.0524 — .0547
		Mean	.0310	.0075		.0536
1.0	2	4.17	.0350 — .0312	.0083 .0112 .0133	.0133	.0580 .0536 .0569
		Mean	.0331	.0109		.0562
1.0	4	2.50	.0369 .0309 .0370	.0051 — .0096	.0149	.0637 .0556 .0583
1.0	4	3.05	.0350 — .0387	.0072 .0092 .0083	.0137	.0584 — .0582
		Mean	.0395	.0079		.0588

60–80 embryos. The range of variation was consequently less, but analytical errors were, for some undetermined reason, larger than in the later experiments. For these reasons the correlation fell to 0.624 with $P = 0.07$ approx. ($n = 7$). The nine values were obtained by pooling results for the second and fourth days, and such correlation as exists is mainly due to the simultaneous fall of respiration and ester content

between the two occasions. The loss of esters is significant (see Table V), and is associated with severe carbohydrate starvation (A. L. James, 1938). It is thus harmonious with the loss of resistant esters observed in whole seedlings between the fifth and seventh days (Arney, 1939).

Table V. *Mean content of esterified phosphate in mg. per embryo (from Table IV)*

Treatment	2nd day	4th day
Full phosphate	0.0109	0.0078
0.2 phosphate	0.0095	0.0075
0.0 phosphate	0.0092	0.0052

Analysis of the variance shows a significant loss between the second and fourth days. In the comparison of occasional with residual variance $Z = 1.663$ (at $P = 0.05$, $Z = 1.459$). The apparent increase with treatment is not significant.

DISCUSSION

The participation of free phosphate, in the production of carbon dioxide from sugars, is indicated by several of our results. Inorganic phosphate has invariably been found in relatively large amounts in all the tissues examined. These include the embryo itself, and the roots and shoots of seedlings taken separately and together. The inorganic phosphate often accounts for more than half the total phosphate (Tables I, II). It has also been shown to increase as a result of phosphate feeding, and is evidently normally available within respiring cells. On account of its abundance it would hardly be expected to restrict respiration rates and we have, in fact, found that the respiration of normal seedlings is independent of further additions of phosphate, even though absorption takes place. Similar abundance of internal phosphate is probably the reason why the early workers obtained such conflicting results. Phosphate deficiency may be set up by depriving the young seedling of its endosperm, and the amount of phosphate then available may exercise a marked effect on the rate of respiration (Fig. 2). This is particularly noticeable if sugar is artificially supplied to provide a low phosphate-sugar ratio. Our results (Fig. 3) then show a direct proportionality between the rate of respiration and the amount of phosphate given: they therefore harmonize with the observations of Said and Sankaran (Richards, 1938) on phosphate-starved leaves of the same species.

The participation of phosphate esters in respiration is indicated by the correlation between their concentration and the rate of carbon-dioxide emission shown in Fig. 5. During the fifth day carbon-dioxide emission is at or near its maximum (Fig. 1). Those enzyme systems which have been investigated in our material (amylases and invertases, Hora, 1936; carboxylase, James & Norval, 1938) are present in excess or even at their maximum development; sugars are also abundant (A. L. James, 1938), and so unlikely to be controlling the respiration rate. It is, therefore, not surprising that the effect of phosphate esters becomes most noticeable at this stage. Inorganic phosphate and residual (phosphatide + nucleoprotein) phosphate do not show similar correlations.

The analyses of free and esterified phosphate carried out on 5-day seedlings

(1936 grain) included ten samples raised on a "no-phosphate" culture solution (Table II). Within this series the results show a strong negative correlation between free and esterified phosphate ($r = -0.71$: $P = 0.05$ with $r = -0.63$). This gives good reason to suppose that the formation of the esters is associated with the disappearance of free phosphate within the tissues, i.e. for the occurrence of the reaction $H_3PO_4 + A \rightarrow$ phosphoric esters, A being some phosphate acceptor or acceptors.

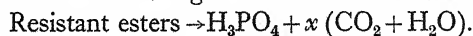
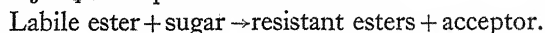
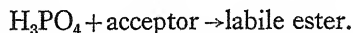
The addition of free phosphate through the culture solution does not necessarily increase the ester content of the tissues; in other words, the natural supply of free phosphate is sufficient to keep the acceptor fully saturated.

Interrelations of the esters. The phosphate esters include a wide range of substances. Of these phosphagens and phytin are absent from our young seedlings (Arney, 1939). Hexose phosphates would be included under "resistant esters" together with such fission products as the triose phosphates, phosphoglycerate and glycerophosphate. It is unlikely that the inorganic phosphate reacts directly with the sugars to give a hexose diphosphate, and this assumption has had to be abandoned for other classes of material. It seems more reasonable to suppose that esterification takes place in our material also through some labile phosphate carrier. The presence of such a carrier ("labile ester") in our seedlings is indicated by the invariable increase of free phosphate on mild hydrolysis of the fraction precipitated by calcium (Arney, 1939). Since this phosphate is split off by boiling with $N HCl$ for 7 min., the labile ester is quite likely to be adenylypyrophosphate, or some similar compound, and the free acceptor adenylic acid. For the present it is necessary to retain the indefinite terms labile ester and acceptor. The concentration of labile ester is usually only a small proportion of the total esters (Arney, 1939), as would be expected if it readily passes its phosphate on to sugars. The low concentration of labile esters found also agrees with the conclusion of the previous paragraph that the acceptor present, being readily saturated, must also be limited in amount.

Between the fifth and seventh days when carbohydrate starvation first begins to be felt, the resistant esters begin to disappear (Arney, 1939), supporting the view that they are directly consumed in respiration. There is a similar loss of esters in the detached embryos between the second and fourth days. Since the resistant esters comprise the great bulk of the total esters on the fifth day, the correlation between respiration rate and total esters already emphasized is probably to be explained in the same way, i.e. by the direct consumption of resistant esters.

As the resistant esters and sugars begin to diminish, the labile esters show a significant increase (Arney, 1939). This appears to indicate that the transfer of phosphate from labile ester to sugar is slowing down, since there is less sugar to receive the phosphate, and perhaps a continuing synthesis of acceptor to retain it.

The results of our experiments are, therefore, readily explained by the following cycle of reactions, and give grounds for supposing that such reactions occur in barley respiration:



SUMMARY

1. The respiration rate (CO_2 emission) of barley seedlings germinating in the dark is independent of the amount of phosphate supplied in culture media.
2. The respiration rate of embryos without endosperms was sometimes, but not always, increased by raising the external phosphate supply.
3. If sucrose was added to the culture media, the full dose of phosphate caused a 60% increase in the embryo's CO_2 output.
4. A strong correlation was observed in 5-day-old seedlings between their phosphoric ester content and rate of CO_2 emission. The correlation disappeared with further carbohydrate starvation.
5. The phosphate ester content of excised embryos was examined and found to decrease between the second and fourth days of germination. The CO_2 output declined simultaneously.
6. Increase of phosphate in the culture media apparently caused a slight increase of esterified phosphate in excised embryos, but the increase was not significant. There was no evidence of increase in complete seedlings.
7. In seedlings without external phosphate supply there was a significant negative correlation ($r = -0.71$; $P < 0.05$) between inorganic and esterified phosphate.

These results are discussed in conjunction with those of the previous paper (Arney, 1939). It is concluded that phosphorylation is likely to occur during barley respiration, and a probable series of outline reactions is suggested.

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BEECH MYCORRHIZA: RE-ISOLATION AND THE EFFECT OF ROOT EXTRACTS UPON *MYCELIUM RADICIS FAGI* (CHAN)

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(With 3 figures in the text)

THE study of the mycorrhizal relationships of trees has, in the past, focused attention mainly on the isolation of the fungal associate into pure culture, and the reformation of the association under experimental conditions. Recently the importance of the conception that the soil under natural conditions is a mixed culture of organisms has been appreciated. In such a mixed culture, the activities of one organism are profoundly modified by the presence and activities of the others. These points have been admirably stressed by Jahn (1934) and Burges (1936) writing on mycorrhiza, and Garrett (1934, 1938) and D'Aeth (1939) in reviews dealing with root parasitism and fungal interraction. Moreover, the concept of the rhizosphere as a volume of soil about the root, in which the root exercises both direct and indirect effects upon the soil and its population, complicates the picture of mycorrhiza.

One is, therefore, faced, in the study of mycorrhizal relationships, not only with a main association of two organisms, but also with a population of organisms living within the sphere of influence of, and themselves influencing, the two main protagonists.

The study of the latter becomes no less important, but the problem is much more complex than has hitherto been realized. Moreover, each constant species of the rhizosphere population deserves special study. Attempts must be made to decide whether each is capable of forming the true mycorrhizal association, and also what part its physiological properties may play in the activities of the whole rhizosphere.

It is with these points in mind that this paper is presented. The observations were made on a fungus isolated from the "pyramidal" type of infection, which has been shortly described in a previous note (Harley, 1937).

No conclusive evidence will be presented that this fungus is the causal organism of this type of infection, nevertheless the data will make it clear that its activities must be important to the association.

ISOLATION TECHNIQUE

Attempts to isolate the fungus forming the sheath on beech roots of the "pyramidal" type, have been made over a period of several years. Amongst the fungi consistently obtained was a sterile mycelium of characteristic form. In most

cultures this was rapidly overgrown by other organisms of the rhizosphere, and it required a special technique to obtain it in pure culture.

Preliminary experiments showed that antiseptics with a high rate of penetration, such as alcohol or toluol, or strong poisons such as 1/1000 mercuric chloride, completely killed the whole association. Gentle antiseptics, like thymol, were not effective in preventing the development of rapidly growing organisms. Bromine water (10%) as recommended by La Rue (1937) was found to reduce the number of fast-growing organisms, but most roots so treated produced a variety of fungi when placed on agar plates. Long applications of bromine water frequently killed the whole association.

A technique was, therefore, elaborated by which the infected roots were cleansed by means of sterile distilled water, combined with a short application of 10% bromine water.

Roots were collected from the humus layer of beech woods and placed immediately in sterile containers, and kept moist by sterile damp cloths. The roots were later placed in tubes of sterile distilled water and shaken in a mechanical shaker. They were then transferred to a fresh tube of water and the shaking repeated, after which they were placed in a sterile petri dish. Short sections of root were then clipped by means of sterile lead clips to a length of sterile gut weighted with lead shot. The whole manipulation was done quickly by means of sterile instruments, inside a small glass-topped compartment which had been sterilized with lysol. The gut, and the roots attached, were then placed in the tube of a washing apparatus of original design, and kept shaken in a continually flowing sterile liquid for several hours.

The washing apparatus was constructed as in Fig. 1. The tube *A*, with its inlets *B* and *C*, and its outlet *D* plugged with wool, was sterilized in the autoclave, then clipped into place on the frame *E*, and the side arms connected with the delivery tubes of the reservoirs *F* and *G* containing sterile distilled water and bromine water. The roots, on their gut, were then lowered through the inlet *C*, and left hanging free in the tube *A*. Tube *A* was then filled rapidly with 10% bromine water to a level just below the bend in the outlet *D*. This was allowed to stand for 30 sec., and then the current of distilled water was started. When the level of the liquid came slightly above the bend in tube *D*, a siphon was set up which emptied the tube *A*. By arranging the rate of outflow, by means of a clip, so that it was about twice the rate of inflow, the tube *A* was filled and emptied at regular intervals; the total flow being about 2 litres in 3 hr. The motor *H* was then started and the tube shaken at the rate of approximately 200 beats per min. In this way the meniscus in tube *A* was shaken over the roots as it rose and fell, and the body of the liquid kept stirred by the lead shot and the clips on the gut.

A somewhat more efficient shaking and washing was effected by a modified design of tube *A*. In this a series of hemispherical bulbs were blown at intervals of about 1½ in. along the length of the main tube. This caused the meniscus and liquid to be kept in greater agitation, and a more rapid washing was obtained.

After this treatment the gut, with the roots attached, was removed and placed in

a sterile dish. The roots were cut into small pieces not greater than 0.5 cm. long, by means of a sterile scalpel, and the pieces transferred to sterile medium in petri dishes.

The following media were used:

- (1) Plain agar, 20 g. per litre of tap water.
- (2) Malt agar, 20 g. agar and 20 g. malt per litre of tap water.
- (3) Chan's agar (Chan, 1923) with or without glucose or starch.

On almost all of sixty plates a mycelium of definite constant type was seen to grow out from the fungal sheath of the roots. In many instances this mycelium was rapidly overgrown by common soil organisms, usually of the following genera: *Mucor*, *Verticillium*, *Penicillium*, *Trichoderma*, *Acrostalagmus*.

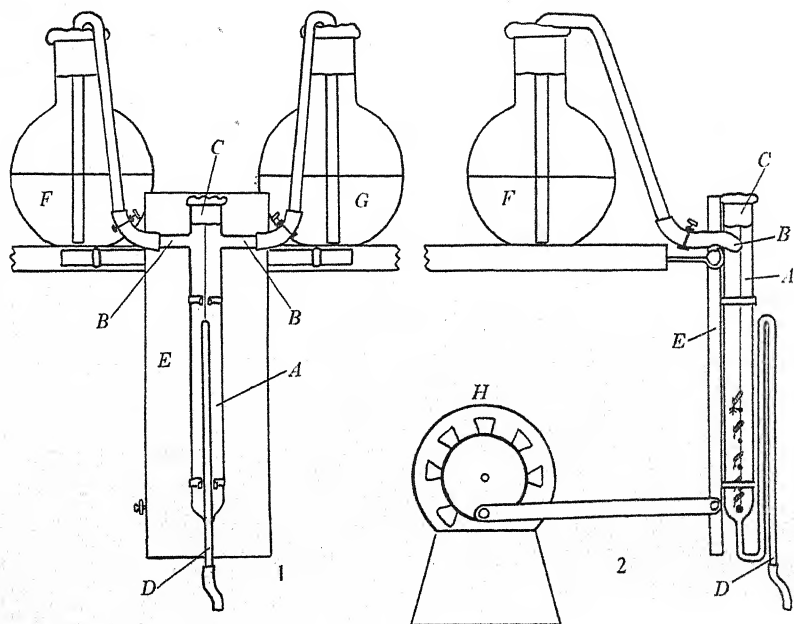


Fig. 1. Shaking apparatus. 1. Face view. 2. Side view. For explanation see text.

But, in several cultures, this mycelium was obtained pure or nearly so. From impure cultures, small pieces of medium (0.4 cm.), containing a few hyphae, were cut out by means of a circular knife¹ and transferred by means of a sterile needle to new culture medium.

In this way many separate isolations of a mycelium which appeared to grow out of the sheath were obtained.

The following is a description of this isolation (Fig. 2 illustrates details): On malt and sugar media the colony in a petri dish is sharply demarcated into regions; a central deep greenish black region, and an outer part of green colour becoming whitish towards the edge. In the absence of sugar, the whole colony remains white,

¹ Designed by Mr G. C. M. Harris, of Oxford.

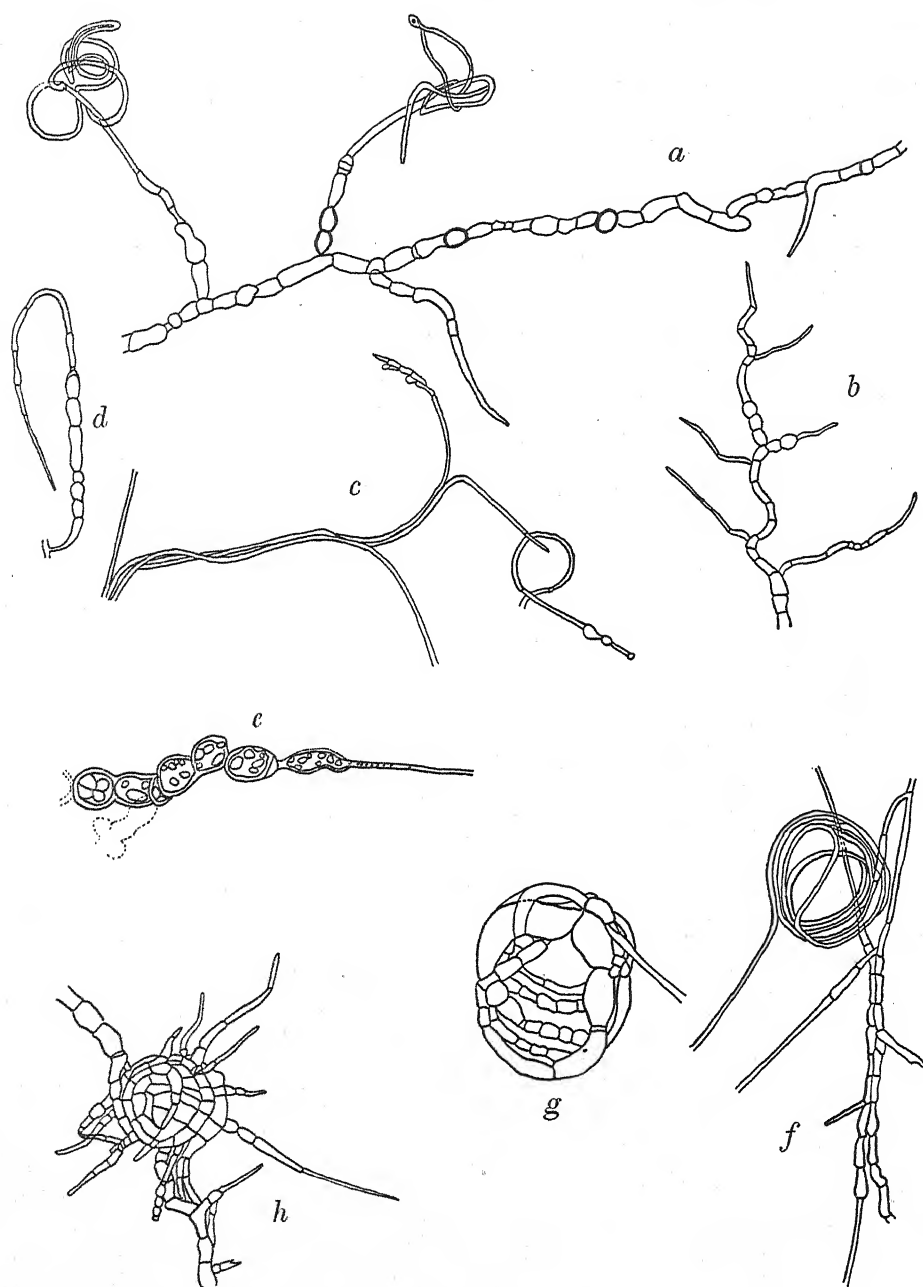


Fig. 2. *a*, Long hyphae of *Mycelium radices Fagi* (Chan) bearing short hyphae, and irregular coils showing septation and conidial formation. *b*, Apex of long hyphae showing slight side to side deviations in growth, conidia, and short hyphae. *c*, Aerial hyphae showing infrequent septation and strands of coherent hyphae. *d*, Conidia upon aerial hyphae. *e*, Conidia from liquid culture, showing thick walls, oil drops, and germination of the apical conidium of the chain. *f*, Coil formed by aerial hyphae upon the surface of the medium in conjunction with surface hyphae. Coil about 60μ in largest diameter. *g*, Details of a typical coil formed upon the surface of agar, 60μ in largest diameter. *h*, Coil, in which the centre is filled with pseudoparenchyma, formed upon the surface of agar medium. Central body of coil about 30μ in diameter.

with a slight greenish tinge towards the centre. On media rich in ammonium salts, the central region is brown, later often becoming black.

The hyphae are septate, with dark pigment, apparently in the walls. No clamps have been observed. The hyphae on and beneath the surface of the agar, are of two types, viz. long and short hyphae. The long hyphae grow continuously outwards from the centre of the colony, and the short hyphae are produced from them approximately at right angles (Fig. 2, *a, b*). The differentiation between the two types of hypha is not sharp, and their diameters grade into one another. Nevertheless the majority of long hyphae are $4-5\ \mu$ thick, whereas the short hyphae are mostly about $2\ \mu$ thick, but both taper to a point.

At first the growth of individual hyphae is not straight. Each tip pursues a remarkable "corkscrew" course, deviating from the straight in one or two planes. This is especially noticeable in the early stages of development (Fig. 2, *b*), and in the young hyphae of slowly growing cultures.

The mycelium readily breaks up into conidia (Fig. 2, *a, b, d, e*), formed in an intercalary, or, less frequently, terminal position by constriction, followed by the formation of walls across the constrictions. The walls frequently become thickened in well-nourished cultures.

Coils of hyphae are sometimes found in old cultures. In some cases these seem to be formed of two or more hyphae together. The early stages compare with the coils described and figured by Noël Bernard (1909) for the endophyte of orchids (Fig. 2, *f*). In the later stages the coils sometimes become filled with tightly packed pseudoparenchymatous tissue (Fig. 2, *h*), and small dark, sclerotium-like bodies are formed.

The mycelium penetrates all layers of the culture medium and also forms aerial hyphae. When growing on deep layers of sugar medium, the greater part of the growth is below the surface. The aerial hyphae are usually thick-walled, dark coloured, and scarcely branched. They are usually less frequently septated than the substrate hyphae (Fig. 2, *c*), but may form conidia (Fig. 2, *d*) and coils in the same way, especially in the centre of the colony. Very often these aerial hyphae, arising from many points in the culture, may come to lie side by side, or become twisted together, forming a webbed aerial mycelium above the substrate (Fig. 2, *c*).

The dark central region of the culture is filled with cellular mycelium (cells $4-6\ \mu \times 4-17\ \mu$) composed of conidia-like bodies. This pseudoparenchyma extends below the surface of the medium, where cells with thick black walls are formed. This central region may be termed a sclerotium. The greater part of the aerial mycelium arises in this region.

This fungus closely resembles *Mycelium radialis Fagi*, isolated by T. A. B. Chan (1923) from infected beech roots in Germany. Table I summarizes the main points of comparison.

Chan claimed that *Mycelium radialis Fagi* was the causative fungus of beech mycorrhiza on the following grounds: that it was frequently isolated from beech roots derived from different sources, that it appeared to grow from the sheath of infected roots, and that the appearance of the central region of colonies on agar

Table I

Character	Chan, 1923	Present isolation (J. L. H.)
Colour	Green, grey-green, black-green, black	Green, grey-green, black-green, black
Colour on aesculin	Dark orange	Medium becoming dark in advance of colony, colony yellow-brown
Septations	Frequent	Frequent
Clamps	None	None
Anastomoses	Especially in old cultures	Especially in old or poorly nourished cultures
Conidia	Formed by constriction	Formed by constriction
Hyphal diam.	Long, 4-6 μ Short, 2-4 μ	4-5 μ 2 μ + } Smaller in poor cultures
Aerial hyphae	Especially on malt and plum agar	Especially on malt and prune agar
Coils	Not described	Especially on poor media
Centre of colony	Black, sclerotium-like	Black, sclerotium-like
Diam. of colony in 10 days on agar	c. 10 mm.	c. 10 mm. Greater lateral spread in absence of carbohydrate
Optimum temp. by lateral spread on agar	?	20-22.5° C.
Upper temp. limit by lateral spread on agar	?	27.5-30° C.

resembled the sheath tissue of mycorrhiza. He admitted that this evidence was not perfectly conclusive, and that the demonstration of reinfection of the roots and the synthesis of mycorrhiza was essential.

Experiments on reinfection are now in progress with this isolation, and it is hoped that the results will be available shortly. The following experiments add to the knowledge of the fungus in pure culture.

GROWTH OF THE FUNGUS IN PURE CULTURE

Chan found that dextrose was a suitable medium for the growth of *Mycelium radicis Fagi*, and it also proved suitable for our own isolation from beech roots. Preliminary experiments were, therefore, set up to test the availability of various nitrogen sources on an agar medium of the following composition

Glucose	50 g.	Agar	20 g.
KH ₂ PO ₄	1 g.	Water	1000 ml.
MgSO ₄	25 g.		

Table II gives the details of growth as measured by increase in the diameter of the colony. The results are not, of course, of great value, but show that all the sources tried will support growth and that, over a short period, nitrate, ammonia and peptone were the best, but that later a slowing of growth occurred on the ammonia medium.

These results were followed by experiments in liquid culture, where it was found that growth was exceedingly slow, especially in the presence of asparagine or monosodium glutamate. Moreover, the mycelium broke up into conidia very readily, and the whole colony became black in colour.

Table II. *Mean lateral expansion on agar (mm.)*

Source of nitrogen	Days from inoculation			
	5	6	11	26
Nitrate	7.0	8.4	10.3	20.0
Ammonia	7.0	8.0	9.2	12.0
Peptone	8.5	9.4	12.1	22.0
Asparagine	5.4	6.4	9.8	18.6
Mono-sodium glutamate	6.4	7.6	9.1	14.7

The outstanding features of the results of the work with pure cultures were the extreme slowness of growth, the inability to attack amide or amino nitrogen with any readiness, and the very slow respiration rate as measured by carbon-dioxide emission. This inactivity was quite unexpected in a fungus as widely distributed in soils usually poor in simple nitrogenous substances, as this appeared to be from the work of Chan (1923) and from these present observations.

It seemed probable, therefore, that some substance that is present in the rhizosphere of beech is lacking from the media used. With this possibility in mind, water extracts were made from beech roots, and their effects on the growth of the fungus tested.

THE EXTRACTION METHOD

The material, consisting of young roots, was washed free of soil in tap water, dried with a cloth, and cut up into short lengths. It was then transferred to a mortar and pounded. A small known quantity of distilled water was added, and the material further pounded. Clean sharp sand was then added, and the grinding continued for 10 min. The whole was poured into a Buchner funnel, and dried with rapid suction. The grinding was repeated with three, or rarely four, further quantities of water, the material being dried on the filter after each grinding.

The whole of the filtrate so obtained was filtered again through a Buchner and then through a bacterial filter. The latter was a "Technico" white-metal filter, fitted with 3 cm. Seitz E.K. asbestos filter films.

A clear yellowish extract was obtained, and was stored in a refrigerator until used.

Table III gives the origin of each extract:

Table III

Extract	Root material	Weight or description	Origin of soil, etc.	Water used (c.c.)
1	Roots of 3-year seedlings	Branch roots of 25 seedlings	Calcareous grit soil	200
2	Roots of adult trees, largely uninfected	40 g. fresh weight	Botanic Garden, Oxford	150
3	Ditto	20 g. "	"	100
4	Young infected roots of adult trees	30 g. "	Bagley Wood	125
5	Old infected roots	50 g. "	"	300

PROPERTIES OF THE EXTRACT

1. Yellow-brown colour, deepening and becoming more reddish on incubation.
2. Complete sterility when incubated at 22° C. for 4 weeks, with or without medium added.
3. Nitrogen, 0.20-0.05 mg. per c.c.
4. Carbon, c. 2.80-0.50 mg. per c.c.
5. Oxidase activity, shown by blue colour in the presence of guiacum.
6. Blackish green colour with ferric chloride.
7. Dark precipitate with dichromate solution.
8. Yellow precipitate with bromine water.
9. Red-brown ring with conc. sulphuric acid.
10. pH 6.9. Minimum buffer capacity between pH 5.0 and 6.0. No precipitation on the addition of acid or alkali.
11. Deepening of colour at alkaline pH, and yellowing at low pH values.

These properties indicate that the extract is a mixture of substances containing, very probably, a catechol tannin and oxidase enzyme system. The small content of carbon and nitrogen is important, since they appear to be too small to act as a substrate for the fungus.

THE EFFECT OF THE EXTRACT ON THE GROWTH OF THE ISOLATION

Liquid media were prepared, having the same constitution as that used above: as carbohydrate source, glucose, and as nitrogen source, nitrate or asparagine were added. To tubes of 5 c.c. of this medium were added either 1 c.c. of extract or 1 c.c. of distilled water. In the first experiment, half of the tubes to which extract had been added were reautoclaved before inoculation. As inocula, circles of agar, 0.4 cm. in diameter, were used. These were cut from the peripheral zone of a culture growing on agar in a petri dish.

The growth was estimated subjectively, and marks were given for the relative amounts of mycelium in each tube. Estimations were made daily, and as often as possible the results were checked by other observers, to whom the conditions of culture were unknown. In each case these observers recorded greater differences between the treatments than the writer. The results of the latter are shown in Fig. 3, *a, b, c*. It will be seen that in each case, growth was increased by the addition of the extract. Stimulation of growth in the reautoclaved tubes was less than that in the untreated ones. Autoclaving resulted in a slight change in the colour of the extract to a redder tinge, and in a loss of oxidase activity. The other properties mentioned above remained.

Many similar experiments gave the same results. Two examples are given in Table IV, showing the final subjective estimations compared with a dry weight measurement.

Table IV

		+ Extract	- Extract
1. Nitrate medium	Subjective estimate	38	19
	Dry weight (g.)	0.0740	0.0699
2. Asparagine medium	Subjective estimate	26	9
	Dry weight (g.)	0.0472	0.0425

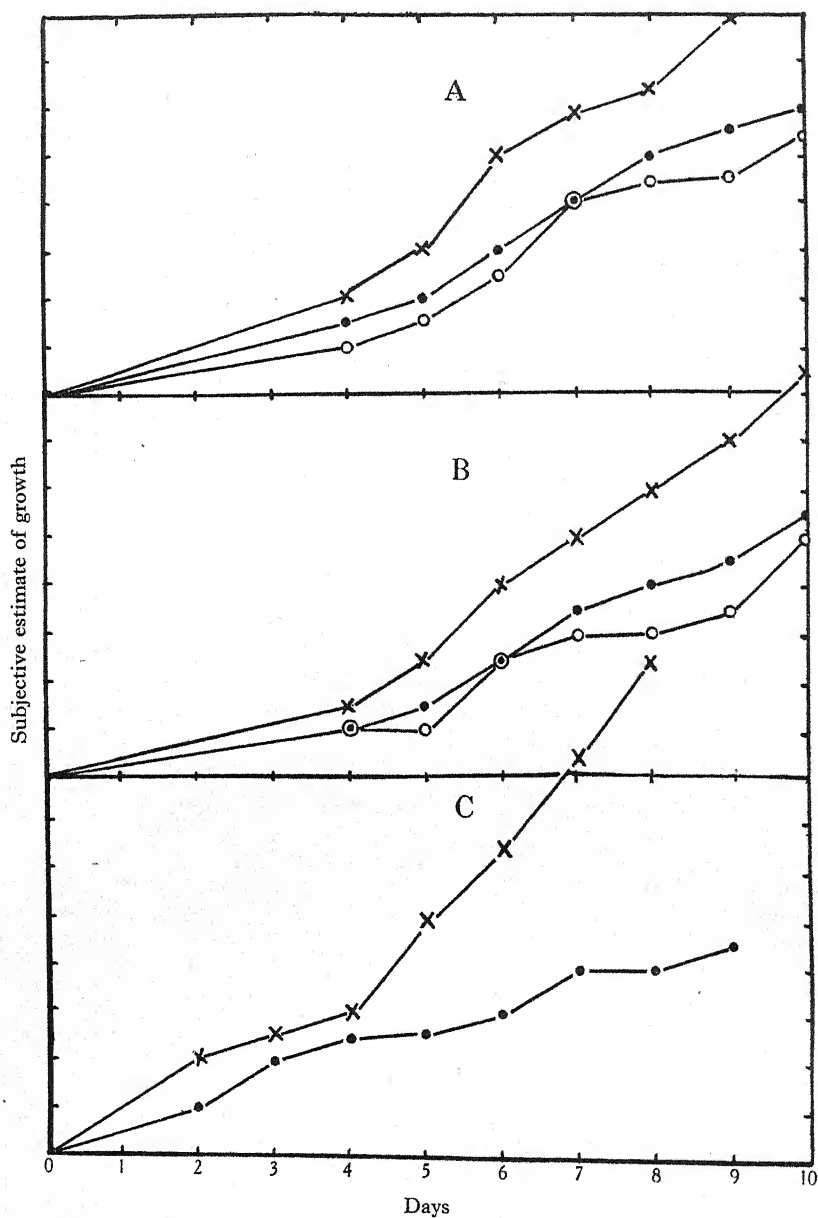


Fig. 3. Growth of *Mycelium radialis Fagi* (Chan) (subjective estimate) plotted against time in days.
 A, Asparagine medium; ○ without extract; ● with extract added before autoclaving; × extract added after autoclaving.
 B, Nitrate medium symbols as in A.
 C, Nitrate medium; ● without extract; × with extract added after autoclaving.

The weights of the colonies from tubes receiving extract are greater than from those receiving none. The discrepancy between the proportions of the weights, and the proportions of the subjective figures is not significant on account of the method of inoculation.

More reliable objective data were obtained by a modification of the method of inoculation. Colonies were grown on very thin layers of plain agar medium in petri dishes. This medium consisted of 1.5% agar in distilled water. This ensured reduction in weight of, and absence of nutrient material from the inoculum.

By this means the effect of the concentration of the extract was investigated. Sets of tubes of liquid medium were made up under sterile conditions, each set containing in 10 c.c., proportions of extract varying from 0 to 6 c.c. After inoculation these were incubated at 22° C. for 25 days. At the end of this time the growth was estimated subjectively and by weight.

Table V

Proportion of extract	0	1/10	1/5	3/10	3/5
Subjective estimate	0	8	9	9	10
Dry weight	0.0042	0.0182	0.0209	0.0168	0.0219
Colour	Black	White becoming black	White with brownish tinge		

As may be seen, a great stimulation was obtained using a medium containing only 1/10 by volume of extract. Further increases in the amount of extract present affect the growth very little, and the evidence for further stimulation is not convincing.

The mechanism of this stimulation, which amounts here to a minimum of 400% and a maximum of slightly over 500%, is unknown, but certain experiments throw some light upon it.

In the first experiment described above, it was noted that, in the presence of the medium, the extract appeared, according to subjective data, to be unstable to heat. Quantities of extract alone were, therefore, subjected to various heat treatments, after which aliquots were added to media, and the rate of growth of the fungus upon them compared with a distilled water control. The results are given in Table VI.

Table VI

Heat treatment	Untreated	40° C.	60° C.	80° C.	100° C.	Auto-claved	Control
Time of treatment	—	1 hr.	1 hr.	1 hr.	1 hr.	20 min.	—
Subjective estimate of growth	17	16	14	15	12	10	4
Weight of mycelium	0.0162	0.0117	0.0122	0.0132	0.0113	0.0107	0.0020
Oxidase activity	+ve	+ve	+ve	-ve	-ve	-ve	-ve

There is no doubt from these results that the most vigorous of the treatments do not destroy the stimulant, and the evidence that they significantly reduce its activity is not strong. One can safely say that the stimulation is not connected with the oxidase activity of the extract as tested by the blueing of guaiacum solution. It is evident that the stimulation amounts to over 500% at the least, and about 800% at the most, in those solutions which contain 1 c.c. of extract 3, per 5 c.c.

Purification of the extract by means of kaolin did not destroy the stimulating property. Aliquots of 25 c.c. of extract 5, were treated as follows:

1. Refiltered through a Seitz filter pad.
2. Treated with Kaolin in the cold for $\frac{1}{2}$ hr. Centrifuged and refiltered through a Seitz pad.
3. Boiled rapidly for 3 min. with kaolin. Allowed to stand $\frac{1}{2}$ hr., centrifuged and refiltered through a Seitz pad.

1 c.c. of each solution was added to a set of tubes of medium and inoculated. Qualitative and quantitative tests were performed on the rest of the extract. The results are given in Table VII.

Table VII

Treatment	Control	Refiltered	Kaolin in the cold	Kaolin and boiling
Weight of mycelium	0.0053	0.0134	0.0141	0.0139
Oxidase activity	-ve	+ve	+ve	-ve
With ferric chloride	—	Greenish black	Greenish black	Greenish black
With sulphuric acid	—	Reddening	Reddening	Reddening
Colour	—	Red-brown-orange	Red-brown-orange (lighter than 1)	Pale orange brown
mg. nitrogen added in 1 c.c. extract	0.0	0.07	0.07	0.05
mg. carbon added in 1 c.c. of extract	0.0	0.50	0.50	0.30

The stimulation in the sets receiving the extract, treated or untreated, was of the same order, and amounted to about 250% at the least, and was in no way correlated with the presence or absence of oxidase activity, which was destroyed by heat in the treatment with kaolin and boiling. The loss of colour in this latter treatment was very marked, and was paralleled by loss of carbon and nitrogen. In all cases, however, the amount of nitrogen and carbon added in the extract was very small compared with the total carbon and nitrogen in the medium, which was approximately 7 mg. nitrogen and 100 mg. carbon per tube (5 c.c.). It seems probable, therefore, that the extract cannot act as a source of nutriment in the ordinary sense, but must provide some form of relatively heat-stable accessory food factor. There is need of course for further elaboration of these points, and to test the effect of similar extracts on other fungi of the rhizosphere of beech. This is being undertaken in conjunction with the investigation of the possibility that *Mycelium radialis Fagi* (Chan) is the causative fungus of a beech mycorrhiza.

SUMMARY

1. A technique for cleansing the surface of the roots of beech is described.
2. A fungus isolated from the "pyramidal" type of beech mycorrhiza is described.
3. This fungus is shown to be extremely similar to *Mycelium radialis* Fagi (Chan), and may be considered to be of the same species.
4. The slow growth of this fungus in pure culture is noted.
5. Growth was found to be stimulated by an extract of beech roots, for which a method of preparation is given.

I wish to thank Dr W. H. Wilkins for advice and criticism, and Mr A. H. Cripps for technical assistance in the construction of the apparatus.

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STUDIES OF THE POST-GLACIAL HISTORY OF BRITISH VEGETATION

V. THE SHROPSHIRE AND FLINT MAELOR MOSSES

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(With Plate IV and 15 figures in the text)

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INTRODUCTION

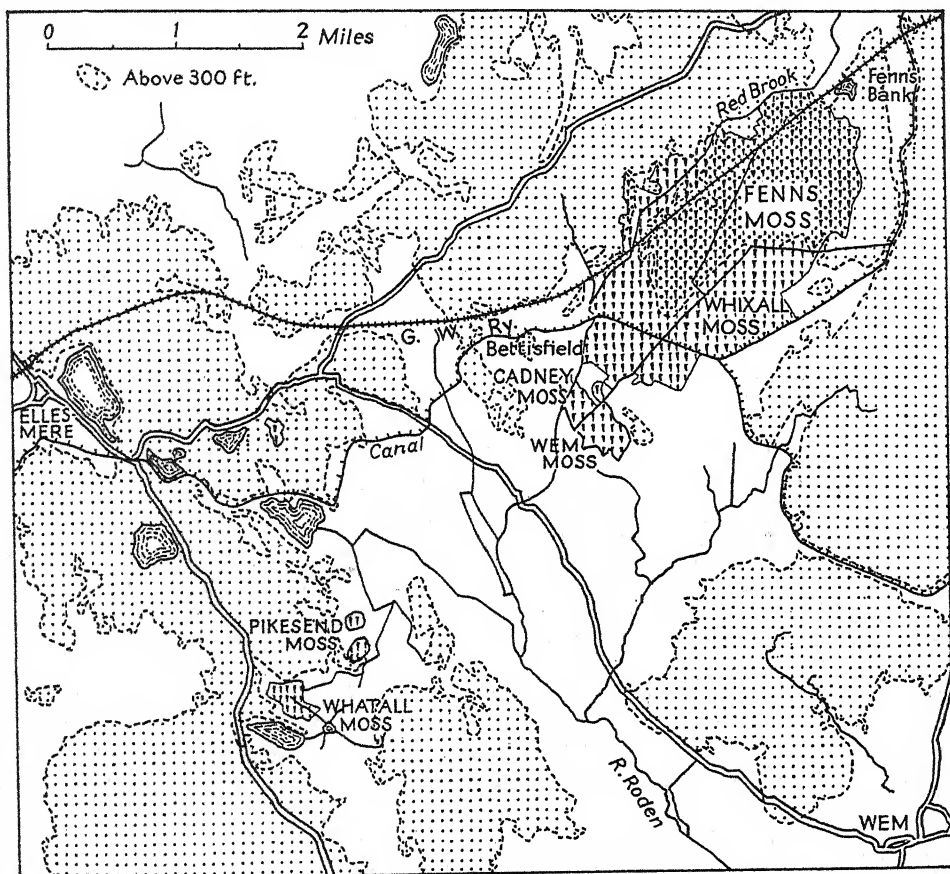
THE northern part of Shropshire is a district of low relief, averaging 300 ft. above O.D., and forming geographically an outlying part of the Cheshire plain.

It is covered for the most part by glacial drift of gravel, sand, and boulder clay. To the south of Ellesmere towards Cockshutt is a belt of country with steep hummocks of sands and boulder clay thought to be a medial moraine formed at the confluence of the north and north-western ice stream with that from Wales. To the east of Ellesmere is another, but rather less steep, belt of moraine probably laid down during the retreat northwards of the ice from the Irish Sea.

In this district around Ellesmere (Text-fig. 1) are a great number of meres and hollows in which peat mosses have formed.¹ These mosses were almost all drained during last century, and few are growing at the present time. Since the draining of the land the margins of the bogs have been obliterated by planting and cultivation so that their original configuration is no longer discernible, and a "lagg" can seldom be seen. Many of them bear trees of pine, spruce, birch, or alder, with an undergrowth of bracken. Others that are wetter bear ling or sweet gale, and some growing *Sphagnum*. But the surface vegetation is everywhere artificial, for even if a moss has not been completely drained, its growing surface has mostly been spoiled by turf cutting. Yet it is evident from their topography and structure that these mosses are the remains of former raised-bogs, although no example of the surface with pools and tussocks typical of a live raised-bog was observed. Along the flood plain of the River Perry, south-west of Ellesmere, there has also been an extensive peat flat known as Baggy Moor, now cultivated: it may have been a fen carr or a raised-bog.

¹ There is some evidence for the existence of an ice-dammed lake over the whole of this area below 300 ft. O.D. during late glacial or perhaps interglacial times. See the *Geological Survey Memoirs* (1925) from which the geological information above is taken.

The draining of the mosses brought to light a number of archaeological objects, but unfortunately either the site of the find was not exactly recorded or the object was too carefully cleaned for any trace of peat to be left on it, so that most of the finds are useless for dating the peat strata. However, owing to the careful work of Miss Lily F. Chitty, F.S.A., full details were available of two sites; these have been investigated, and the results are described here.¹



Text-fig. 1. Map of the district between Ellesmere and Wem showing the general topography and some of the mosses and meres.

I should have been unable to carry out this work without the help and advice of Dr Godwin and Miss L. F. Chitty, F.S.A. Miss Chitty kindly introduced me to the region, advised me in the choice of sites, and has constantly helped me with her great knowledge of the locality and its antiquities. Dr Godwin has guided the whole course of the work, and given me the benefit of his help and experience with the field work and with every problem that arose. No one could have been more generous than they, and I wish to thank them most sincerely.

¹ Among other moss-finds, which could not be made use of for the reasons given above, are: a socketed bronze axe, a bronze shield, both from Hordley; a water-clock from the Berth, Baschurch; and a bronze spearhead from Ruyton Moss.

I am greatly indebted to the Managers of the Scandinavian Fund in the University of Cambridge and to Newnham College for the generous studentships which enabled me to study the methods of pollen-analysis in Sweden and to carry out this work in Cambridge; and especially to Prof. von Post who first instructed me in the technique of pollen-analysis at the Geologiska Institut of Stockholms Högskola, and so kindly and patiently explained his work to me and allowed me to take part in it.

My thanks are also due to the Manager of the Moss Litter Works at Bettisfield, Miss Alison Clay, Mr H. L. Edwards, Sir Cyril Fox, Mr H. J. E. Peake, Mr George Saywell, Dr Hamshaw Thomas, and to the Shropshire Archaeological Society for the loan of the blocks for Text-figs. 3 and 8, and to the Director of the Geological Survey and the Controller of H.M. Stationery Office for kindly allowing the reproduction of the photographs in Plate IV.

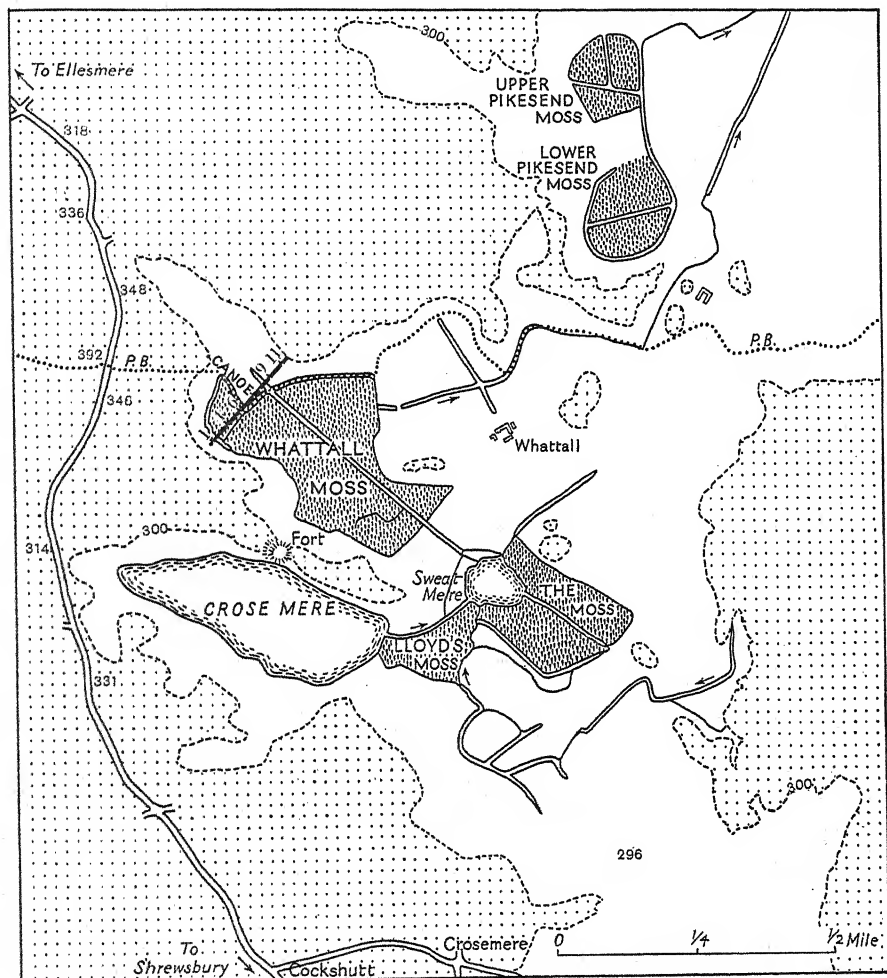
WHATTALL MOSS

This moss (Text-fig. 2) is situated 3 miles south-south-east of Ellesmere. It lies about 285 ft. O.D. in a narrow gully between two steep ridges of glacial gravel which rise to nearly 350 ft. The south-western ridge juts out like a peninsula dividing the moss from Crose Mere, an open stretch of water joined to Lloyd's Moss and Sweat Mere at its eastern end. On this ridge between Whattall Moss and Crose Mere is an earthwork marked as "Fort" on the Ordnance Survey maps. It consists of a bank across the ridge with a ditch on each side. Some excavations were carried out here nearly fifty years ago (Peake, 1909), but no datable material was found, and there was no definite evidence of the date of the structure. However, the result of the excavation "showed us that at some former time a ditch had been dug connecting Crose Mere with the sheet of water which formerly occupied the site of Whittall (*sic*¹) Moss, and that this ditch had contained water was further demonstrated by a layer of sand at its bottom, similar to that in the bed of the mere, and further, because in this sand were embedded the roots and the lower portions of the stems of some large reeds" (Peake, 1909). At that time the level of the mere must have been about 8 ft. higher than at present. Under these conditions the western part of the peninsula would have been an island protected by water or boggy ground on all sides. Plate IV *a* shows the character of the country at the present day.

It is clear that in former times when the water-level was higher the meres extended farther in all directions, and the area between Lloyd's Moss and Whattall Moss where the gravel of the valley floor rises, must until recently have been occupied by wet and boggy ground as it is covered by a thin blanket of peat, though moss no longer grows there. Probably in earlier times both Crose Mere and Whattall were part of one large lake. At the present time the upper end of Whattall is dry and cultivated, but a darker soil in the bed of the valley indicates the former extent of the water. What remains of the moss is enclosed by drainage ditches. Approaching from the east, on the north side of the parish boundary between Cockshutt and Ellesmere parishes, the surface is fairly dry, birches growing to a height of about 25 ft., and abundant *Pteridium* below. Farther westwards it becomes increasingly

¹ This is an alternative spelling which occurs on some maps.

wet, with *Myrica gale*, tussocks of *Sphagnum cymbifolium*, and more alder among the birch trees. This vegetation ends abruptly at the western fence against the steep slope of the gravel ridge. South of the ditch cut along the parish boundary it becomes increasingly drier, with more eutrophic species, *Pinus sylvestris* growing to some



Text-fig. 2. Whattall Moss and surroundings. For the most part the mosses are now quite dry and planted with trees. The site of the dug-out canoe and the line of the section shown in Text-fig. 4 can be seen at the north-western end of Whattall Moss. The dotted line P.B. marks the parish boundary between Ellesmere and Cockshutt parishes. Land above 300 ft. O.D. stippled.

40 ft. in height, and the moss peters out against a slight rise of gravel on which oak and elm are growing. Only to the east of the main north-west-south-east drainage ditch is there much depth of peat in the Cockshutt portion. Here, in an area whose surface consisted of dry crumbly peat bearing *Pteridium*, a boring was made at a

point approximately 60 yards west-north-west from the gate on the footpath, and close to a belt of birch trees and rhododendrons: the stratigraphy recorded was:

cm.	
0-138	Dry dark chocolate-brown structureless peat, H 8-9. Some fragments of wood.
138-175	Yellow-brown sedge peat with fine yellow rootlets much humified.
175-235	Sedge peat, seeds of <i>Menyanthes</i> , H 4-5.
235-247	Gyttja. ¹
247-255	Transition, fine sand, tiny pebbles and seeds.
255-270	Gyttja.
270-	Clay and pebbles.

In 1864 extensive drainage operations were undertaken, and the moss brought to its present condition; the level of Crose Mere is said to have been lowered by 6-10 ft. In cutting the drain along the parish boundary where it crosses the moss, a dug-out canoe² of oak (Text-fig. 3) was found lying across the trench, at a depth of about 6 ft. from the surface. The accounts of the find are confused and contradictory, but Miss Chitty has recently surveyed the evidence and states (Chitty, 1927, pp. 116-17):

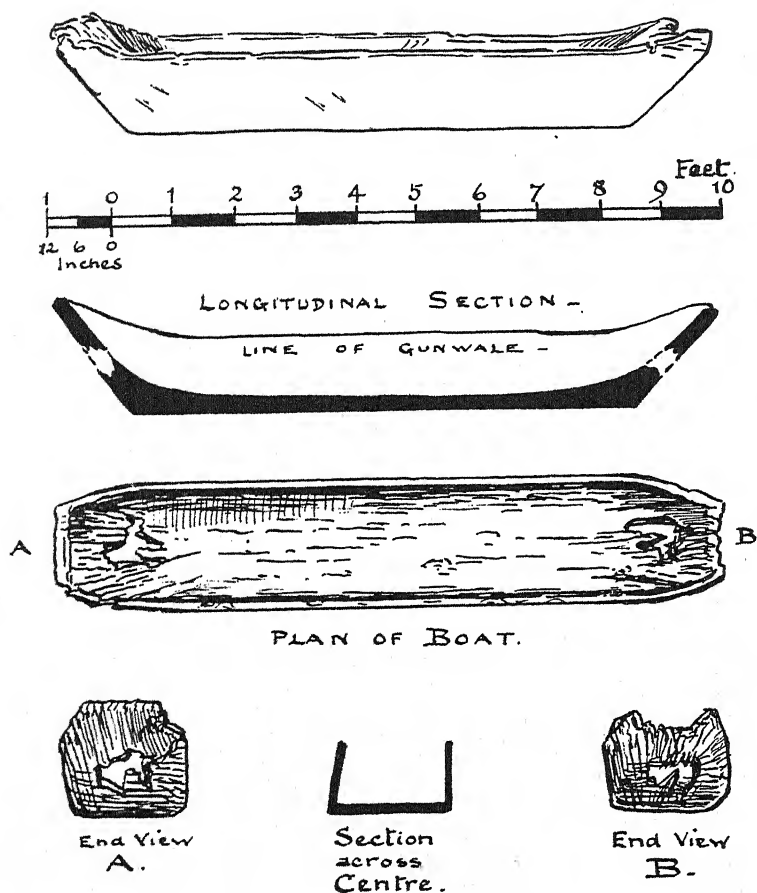
At a depth of about 6 ft. a dug-out canoe (Fig. 1)³ was discovered buried in the peat and enmeshed in the roots of a large birch tree, which had grown through both its ends. . . . It is an admirably finished example of the trough-like or punt type of boat, with squared sides and ends and a flat bottom: Dr Fox describes it as a variant of his Group I, "resembling a canal barge with overhanging counters". It is 10 ft. 9 in. long, 2 ft. wide and 1 ft. 2 in. in height amidships: the sides are straight for a length of 7 ft. 6 in. thence incurving gently to an approximately straight-ended prow and stern, 1 ft. 3 in. and 1 ft. 4 in. wide respectively. The prow (*A* on plan) is 1 ft. 10 in. from ground-level, 3 in. higher than the stern (*B*), the centre of which is lower than its sides, probably owing to damage by the birch roots, which have injured the wood and left jagged rents in the slopes at both ends. The starboard has warped inward slightly, but the original section was probably truly square. The floor is flat within and without, with clean angles along the sides: internally, it curves up to the ends without any line of division: Dr Fox's plan of it applies to the base only. The gunwales are squared off, 1¼ in. wide, broadening towards the ends. Tool-marks are scanty, but a few diagonal cuts with a clean, straight edge on the port side and near the prow suggest that the boat was made with a straight-bladed tool, probably an iron adze: a stone or bronze implement would give a curved cut.

Nothing was found with the canoe except a wooden baler, and there is no evidence for dating her, though Sir Cyril Fox (Fox, 1926) has shown that another of somewhat similar design may have belonged to the Roman period. It is obvious, however, that this vessel must have been in use at a time when there was open water, before moss had grown over the mere deposits. The exact spot at which the find was made can still be seen where part of the bank on the Ellesmere side had to be cut out so that the canoe could be removed. A boring was made 3½ ft. west of the

¹ The Swedish word "gyttja" is used throughout to denote organic or nekron mud formed of the remains of plants and animals, mostly plankton. It is equivalent to "nekron-mud".

² Now in Shrewsbury Museum; cf. *Trans. Shrops. archaeol. nat. Hist. Soc.* 4th Ser. 12 (1929), 68.

³ My Text-fig. 3.



BOAT FROM
WHATTALL MOSS,
ELLESMERE.

Lily F. Chitty -
1926.

Text-fig. 3. Dug-out canoe from Whattall.

side of this bay, and samples were taken for pollen-analysis. The stratigraphy was as follows:

cm.	
0-150	Fresh <i>Sphagnum cymbifolium</i> peat, H 1-3, roots of <i>Oxycoccus</i> , <i>Eriophorum angustifolium</i> .
150-160	Transition and lake mud.
160-225	Greyish brown much decayed gyttja peat with abundant moss stems.
225-290	Dark chocolate brown gyttja.
290-320	Lighter gyttja containing white rootlets.
320-370	Green elastic gyttja.
370-390	As before with some <i>Phragmites</i> .
390-445	Green gyttja.
445-505	Coarse detritus gyttja containing some clay.
505-515	Green elastic gyttja.
515-540	Blue clay and pebbles.

A line of borings was made roughly parallel to that of the ditch in which the canoe was found, from the western edge of the moss, across it, and continuing the line eastwards across the meadow to the gravel ridge. The section thus obtained is given in Text-fig. 4. At the eastern side there is a considerable depth of lake deposits right up to the margin, and from there westwards the floor slopes down gradually until from station 6, where the bottom was at 580 cm., there is a steep dip to stations 5 (bottom at 810 cm.) and 4, only 55 yards from station 6, where we were unable to reach the clay with the full extent of the borer at 1350 cm. (about 44½ ft.). At this site, station 4, there was 150 cm. of fresh *Sphagnum* peat at the top; below this there was too much water for the borer to open, but it appeared to be soft gyttja from 300 cm. downwards. From 1300 to 1350 cm. a detritus gyttja layer was reached of which it was possible to take samples for pollen-analysis (Text-fig. 6). At station 3, bottom was not reached at 1250 cm. where there was gyttja.

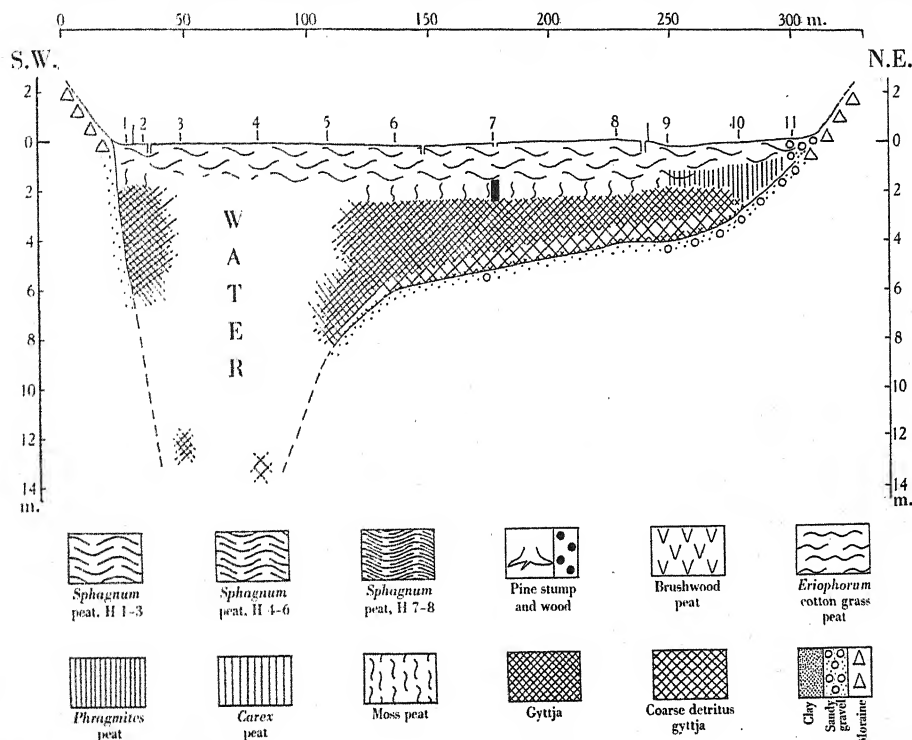
Boring to such depths was a great labour, and it was impossible to do more than prove the existence in this area of a remarkably deep and steep-sided pit which may well have been a kettle-hole.

The series of samples taken for pollen-analysis at station 7 where the dug-out was found yielded the diagram (Text-fig. 5). Pollen was fairly abundant throughout, and 150 grains of tree species were counted in each sample, except that from 530 cm.—the blue clay—it was impossible to make a count of this one even after treatment with hydrofluoric acid; one pine, two birch, one oak, two alder, one willow, and two hazel were seen, but these may possibly be derived grains and not contemporary with the deposition of the clay.

As the greater part of the deposits is lacustrine, the diagram up to the base of the *Sphagnum* peat at 150 cm. should be comparatively free from immediately local influences such as trees and plants growing on the surface, and should therefore reflect fairly accurately the forest history of the neighbouring countryside. From the surface to 150 cm., in the analyses from the *Sphagnum* peat, which supports at

the present day a growth of birch trees, the local characters of the vegetation are probably over-emphasized.

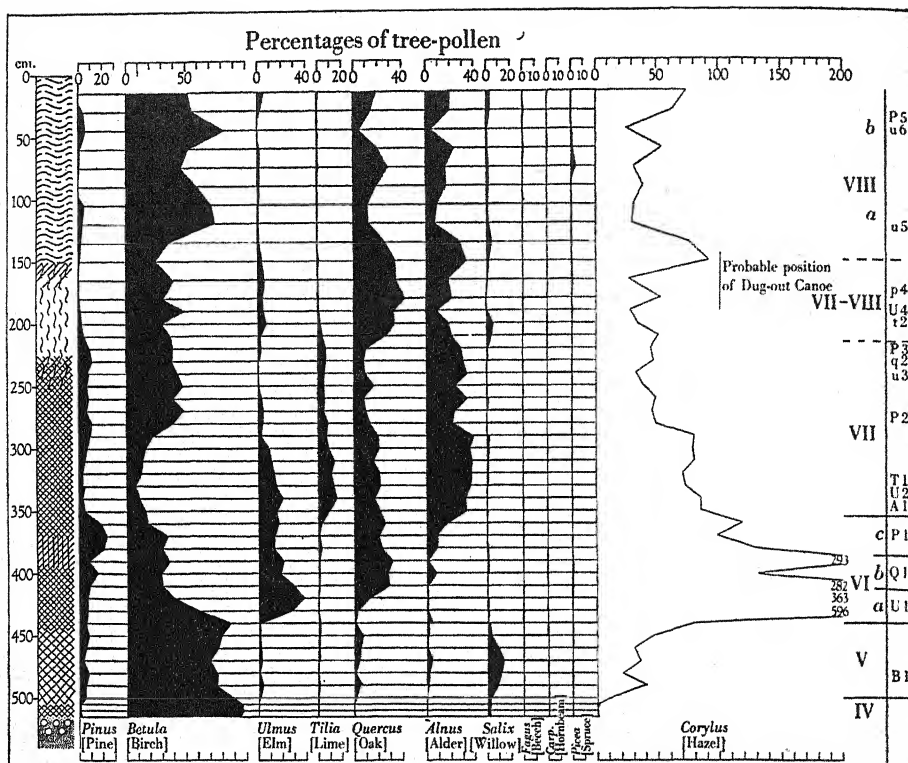
The bottom 15 cm. show an overwhelming dominance of birch with QM.¹ and hazel represented only by an occasional grain. Between 500 and 440 cm. there is a definite increase in QM. and hazel, alder appears, and willow has its largest maximum. 440-360 cm. is characterized by tremendous values of hazel which reaches 596 %. During this stage too there is a rapid development of QM. usurping



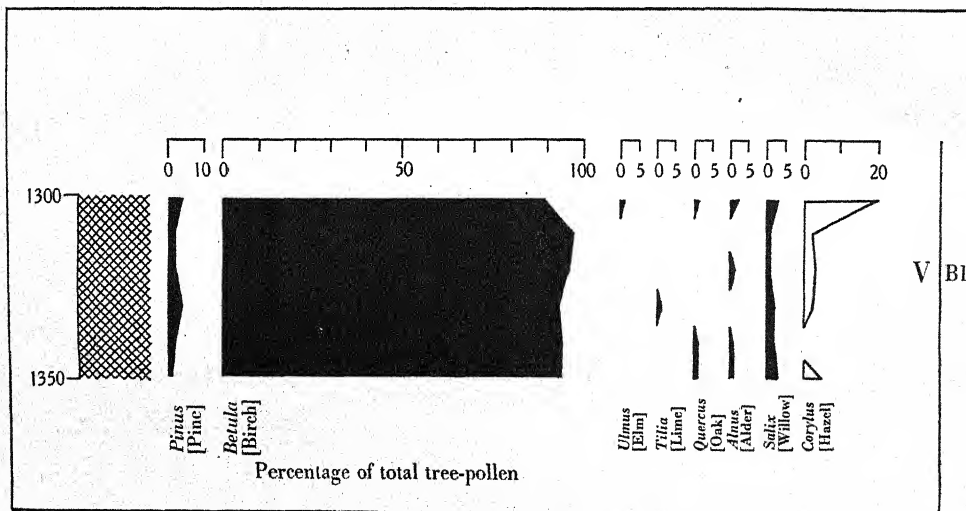
Text-fig. 4. Section across Whattall Moss. Station 7 is the site of the dug-out canoe whose probable position is shown by a thick black line. The deposits in the morainic basin consist chiefly of organic lake mud overgrown by a mat of *Sphagnum* moss. On the south-west side there is much water, and it was impossible to reach the bottom on account of the great depth: it seems likely that this deep pit is a kettle-hole. Combinations of the symbols are not shown above, these are self-explanatory. 'Moss peat' is derived from mosses other than *Sphagna*.

the position of birch. From 360 to 280 cm. alder and lime increase markedly. Above 280 cm. the curves are very variable, but from 150 cm. upwards, in the analyses from the *Sphagnum* peat, local birch is the strong dominant. Notable features are the termination of the continuous lime curve at 180 cm.; two small pine maxima at 280 and 230 cm. respectively, followed by recession corresponding to that of lime; an elm maximum between 190 and 165 cm.; and sporadic appearances of beech, hornbeam, and spruce above 190 cm. The significance of this diagram will be considered below with the others available from this area.

¹ The letters QM. (*Quercetum mixtum*) are used all through to denote mixed forests of oak, elm, and lime.



Text-fig. 5. Pollen diagram and stratigraphy from the site of the dug-out canoe, station 7, Whattall Moss. Hazel is calculated as a percentage of the total tree pollen, its chief maximum (Cr) extends through phases VI (a) and (b). Symbols as in Text-fig. 4. The Roman numerals refer to the zones and the letters and figures to the maxima and minima of pollen (cf. Table I). The horizontal ruling shows each level at which an analysis was made.



Text-fig. 6. Pollen diagram from samples taken at the lowest level reached at station 4, Whattall Moss. The vertical scale refers to the depth in centimetres below the surface. Symbols as in Text-figs. 4 and 5.

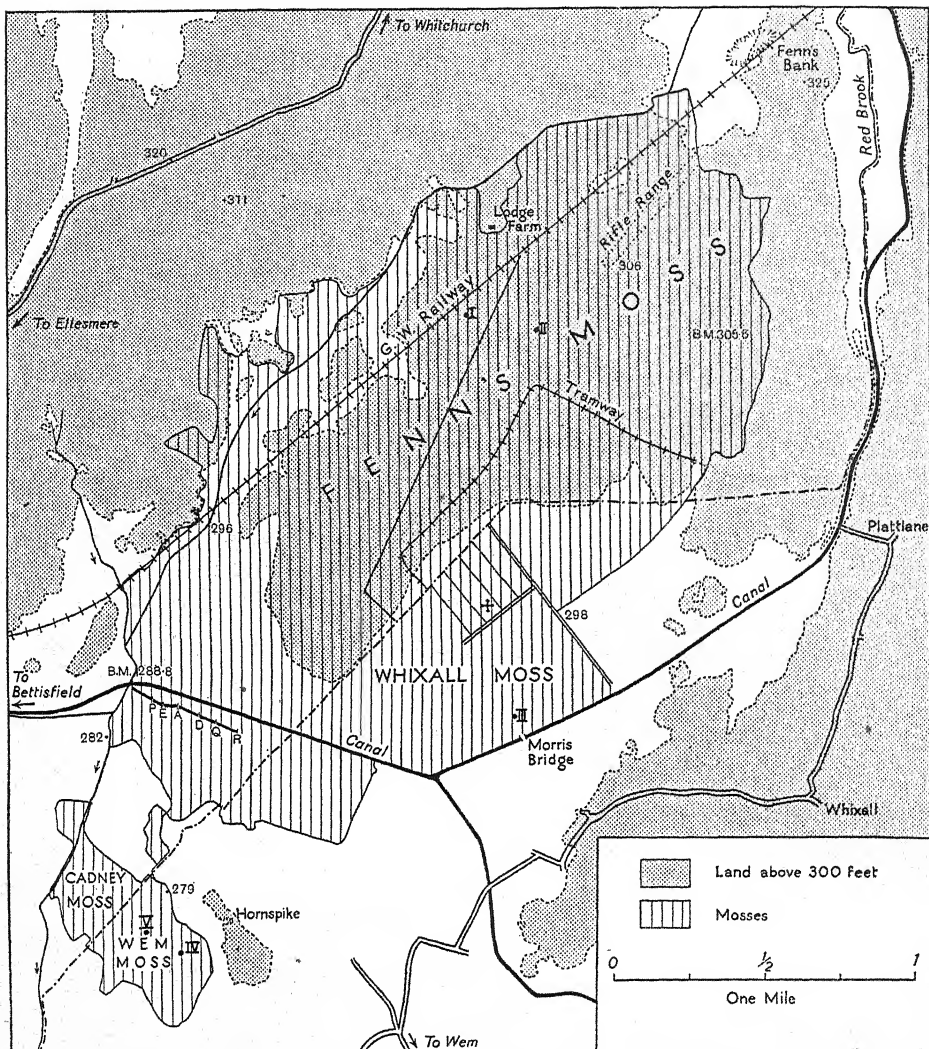
The dug-out canoe is said to have been found at a depth of 6 ft. The water-table is at present about 25 cm. below the surface of the moss, so that one may suppose that there has been some slight shrinkage of the upper part, at least, of the peat, making the corresponding depth at the present day possibly slightly less, but certainly not greater than 6 ft. A level in the diagram below 190 cm. need not, therefore, be considered. On the other hand, it is most unlikely that the canoe reached her position after the mat of *Sphagnum* moss had begun to grow when there would be no open water to float her on, or that she was sunk in a trench, for had she been a votive offering, such as the Hjortspring boat (Rosenberg, Jessen & Johannessen, 1937), deliberately embedded in a *Sphagnum* moss, one would expect to have found gear and accessories. On these grounds it seems reasonable to suppose that the bottom of the canoe lay somewhere between 150 and 190 cm., and is roughly contemporary with this stratum.

WHIXALL MOSS

About 5 miles east of Ellesmere lies a very extensive moss (Text-fig. 7 and Pl. IV *b*) covering more than 5000 acres, with two subsidiary mosses to the south-west. Practically the whole area has been drained, and turf is cut in large quantity, but clearly this moss has been a fine raised-bog. The bog extends for nearly 4 miles in a south-west-north-east direction from Bettisfield to Fenn's Bank; its shape is oval with a maximum width of about $1\frac{3}{4}$ miles, but the margins have been obscured and encroached upon by drainage and cultivation. The county boundary cuts across the moss, dividing the part to the north called Fenn's Moss in Flintshire, from Whixall Moss in Shropshire. Across the south-western half runs the Shropshire Union Canal from Bettisfield to Plattlane; and the Ellesmere-Whitchurch railway line crosses Fenn's Moss. The moss is drained northwards by the Red Brook, which joins the Wych Brook and the Dee, and southwards by the River Roden (Text-fig. 1). A big artificial drain follows the course of the county boundary between Whixall and Fenn's Moss. The 300 ft. contour crosses the middle of the moss with slightly higher levels to the north-east; the old Rifle Range at 306 ft. appears to be about the highest point on the surface which slopes away south-westwards towards Cadney Moss and Wem Moss. In the *Geological Survey Memoirs* (1925, p. 82) it is stated that the surrounding country is Boulder Clay; that the moss rests on a bed of clay of varying thickness; and that peat is cut to a depth of from 16 to 30 ft.—these figures presumably refer to measurements made before the moss was so extensively drained and shrunk as at present. Peat cutting is now most active on Fenn's Moss, where good sections may be seen from 1 to $1\frac{1}{2}$ m. deep. At a point (I on Text-fig. 7) south-west of Lodge Farm, some hundred yards south of the railway, on the western slope of a small relatively untouched area near the higher part of the moss, the following stratigraphy was observed in a drain running roughly north-south:

cm.	
0-10	Surface burnt.
10-29	Very fresh <i>Sphagnum cuspidatum</i> peat, roots of <i>Eriophorum vaginatum</i> and <i>Eriophorum angustifolium</i> , H 1-2.
29	Sharp contact to H 3-4. <i>Calluna</i> and <i>Erica</i> .

- cm.
 29-34 Transition.
 34-115 Dark brown *Sphagnum* peat, H 5-6, *Calluna* frequent. Fresh layers of *Sphagnum cuspidatum* at 75 cm.



Text-fig. 7. Fenn's and Whixall Mosses and surroundings. The site of the palstave from Whixall Moss is marked with a cross (*). The section line across part of the Bettisfield portion may be seen south-west of the canal. Heights above O.D. in feet. The Roman numerals I-V indicate approximate positions of trial borings. The broken line represents the county boundary.

A boring (II on Text-fig. 7) was made on the highest point of this untouched patch, where the sequence obtained was:

- cm.
 0-100 Fresh *Sphagnum* peat, light brown, some *Calluna* and roots of *Eriophorum angustifolium*, and *Eriophorum vaginatum*, H 2-3.

cm.	
100-128	<i>Sphagnum cuspidatum</i> , H 3.
128-150	<i>Sphagnum</i> peat, roots of <i>Eriophorum angustifolium</i> and <i>Eriophorum vaginatum</i> , H 5-6.
132	Fragment of charcoal.
150-240	H 6-7. Roots of <i>Erica</i> and <i>Eriophorum</i> .
240-328	H 4-5. Roots of <i>Eriophorum</i> .
328-342	Wood peat.
342-350	Grey sand.

Another trial boring (III on Text-fig. 7) was made on Whixall Moss just north of the Canal near Morris Bridge. Here the stratigraphy was:

cm.	
50-100	<i>Sphagnum</i> peat, H 6, few roots of <i>Eriophorum angustifolium</i> , some wood and reeds.
100-205	Quantity of fine yellow rootlets, probably <i>Carices</i> , seeds of <i>Menyanthes</i> , W 1.
205-220	Wood peat.
220-240	Grey silty sand.
240-250	Clean sand.
250-300	Stiff pink clay.

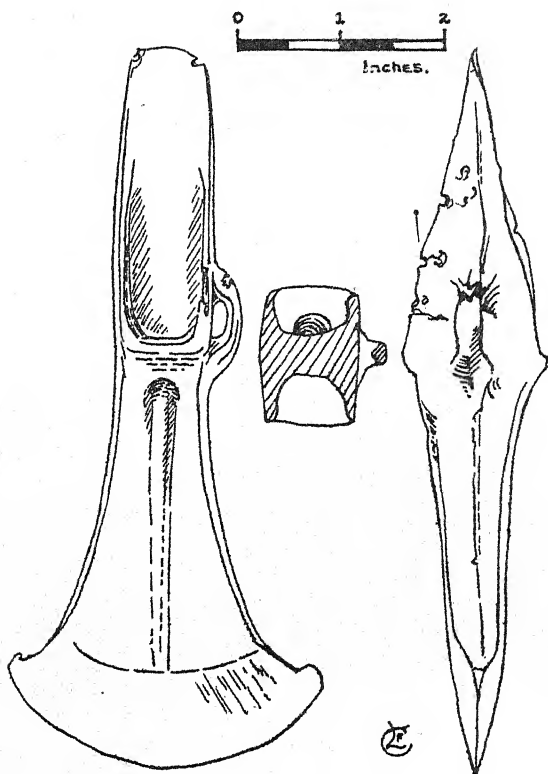
Where cutting has not interfered (as it has in the third section above) this bog shows a well-marked division into an upper unhumified peat, and a lower dark compact humified peat. Such a disposition is common in the raised bogs of north-western Europe, and the sharp boundary between the two types of peat is referred to as the "gräns" or "Grenz-horizont", which is perhaps best rendered in English as "Recurrence surface" or "Rejuvenation surface". This surface is generally held to mark the transition from the dry Sub-Boreal to the cold and wet Sub-Atlantic period. Recently Granlund (1932) has described a number of these recurrence surfaces of different dates within the bogs of southern Sweden, and although the most pronounced of them, RY III, is held to correspond with the Sub-Boreal-Sub-Atlantic transition, his work does raise some doubt as to whether any well-marked recurrence surface in a west European bog need necessarily be of this particular age.

Turf has not been cut recently on Cadney Moss and Wem Moss, and the surface which is pitted with old shallow cuttings in which *Sphagnum* is growing, bears for the most part a rampant cover of *Calluna*, with local patches of *Myrica*, especially on Wem Moss where there are signs of recent burning. Here and there on this moss stumps of large pine trees stand out, their roots creeping into the moss a few centimetres below the surface. One trial boring (IV on Text-fig. 7) was made about a hundred yards from the margin below Hornspike House, and another out in the middle of the moss. At the former there was:

cm.	
0-165	<i>Sphagnum</i> peat, H 5-7, roots of <i>Eriophorum vaginatum</i> . Fragment of charcoal at 125 cm.
165-227	Sedge peat, <i>Phragmites</i> and seeds of <i>Menyanthes</i> .
227-	White sand.

At the second boring (V on Text-fig. 7) a similar sequence was found with bottom at 497 cm.

The most important site to be investigated was the spot where, in 1927, Mr George Saywell of Whixall had found a bronze looped palstave when he was digging turf on Whixall Moss. A detailed description of the find and of Mr Saywell's account has been published by Miss Chitty (Chitty, 1933); but, as this is the only datable archaeological find from peat in this region of which we have any precise



Text-fig. 8. Bronze palstave from Whixall Moss.

and reliable record, it is necessary to repeat the details of it here. The palstave (Text-fig. 8) is a fine specimen $6\frac{3}{4}$ in. long with a maximum width of 3 in.; the butt is thin, there is a pronounced square stop-ridge, and a loop at one side. The form of the blade closely resembles that of a flanged axe, expanding towards a crescentic edge with recurved and almost barbed tips. Down the middle of each face from the stop-ridge to the bevel of the cutting-edge runs a round tapering mid-rib such as is generally thought to be a skeuomorph of the cleft stick in which a flat axe would be hafted. The profile of the palstave is diamond-shaped. The implement is a standard type of the Middle Bronze Age.

Mr Saywell made the find at a place which he described exactly to Miss Chitty (Chitty, 1933, p. 73):

He described and marked the exact site of its discovery on the 6 in. O.S. Shropshire Sheet VII S.W., within the County boundary near the S.E. corner of the map, $\frac{3}{4}$ in. east of the triangulation mark 297 almost due east of Oaf's Orchard (Lat. $52^{\circ} 55' 8''$; Long. $2^{\circ} 45' 20''$). It was found near the bottom of the Lord of the Manor's land, in the third division S.W. from the main drain which runs S.E. from the boundary drain to the Shropshire Union Canal: the spot was in the second pit dug near the eastern angle, about 40 yards from the side drain and 4 yards from the main "casey" where the footpath runs.

He was "nicking out the black turf", and told me that the implement was lying "on top of the roots of the old pine, about 8 ft. from the surface". Unfortunately, no trace of pollen-bearing material from which an analysis could be made remained on the palstave; and, although Mr Saywell kindly allowed me to examine it and scrape all the possible material out of the crevices, none of it contained any pollen.

Mr Saywell also informed me that groups of pine stumps occurred here and there in the peat about 8-9 ft. from the surface. There was no sign of them in the cuttings on the northern part of the moss, but they are to be seen in some places on Whixall Moss lying 3 or 4 ft. from the present surface (the difference in level is no doubt due to the shrinkage of the peat or cutting away of the top layers).

The site of the find was visited in September 1937. That part of the moss is at present devastated after the severe cutting and draining of some years ago. A number of pine stumps stand on the bed of a wide cutting littered with heaps of old sods; along the western side is a narrow bank left uncut which carries a footpath down from the main track. The old surface of the moss on this bank has been worn away, and the peat is dry and shrunken, but it appeared to be the most complete section that remained near to the pine stumps where the palstave had been found.

A boring was made on this bank about 15 yards from the end and 3 ft. in from the edge of the cutting. The stratigraphy is rather similar to that found on the margin near the Canal at Morris Bridge, though the depth of peat is greater and equal to that out in the middle of Fenn's Moss, suggesting that the thickness of the moss is more or less uniform.

cm.	
0-20	Withered and highly humified.
20-55	Fresh <i>Sphagnum</i> peat, H 1-2, some <i>Calluna</i> and <i>Eriophorum vaginatum</i> .
55-140	<i>Sphagnum</i> peat, H 4-5.
140-150	Mat of <i>Eriophorum vaginatum</i> and <i>E. angustifolium</i> , R 3.
150	Fragment of pine bark.
150-160	Transition with <i>Phragmites</i> .
160-180	<i>Sphagnum</i> -sedge peat, <i>Phragmites</i> , and abundant seeds of <i>Menyanthes</i> .
180-190	Roots of <i>Oxycoccus</i> and <i>Eriophorum vaginatum</i> .

cm.	
190-340	Sedge peat, <i>Phragmites</i> , <i>Sphagnum</i> , <i>Menyanthes</i> , becoming muddy towards the bottom with wood fragments below 280 cm.
340-360	Gyttja.
360-370	Blue sandy clay.

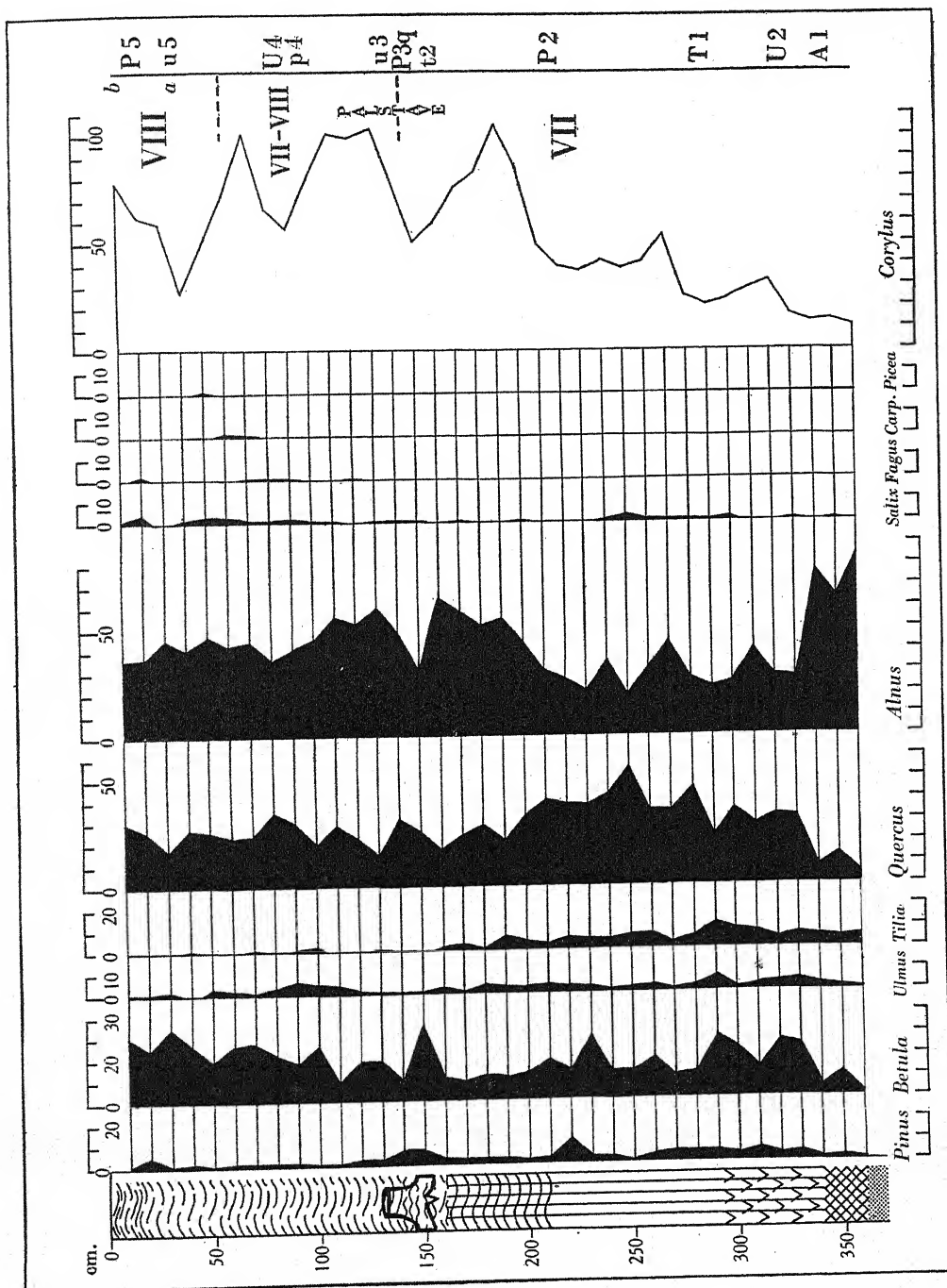
It was difficult in the boring to detect a true "gräns" between the upper and lower *Sphagnum* peats that was not merely the effect of drying and water-level; but, in the section exposed on the side of the bank which had slumped down somewhat through the crumbling away of the older peat, one could observe a definite change at the base of the upper *Sphagnum* layer at 65 cm., which was 25 cm. above the base of the *Eriophorum* layer. The bed of the cutting itself was so weathered that one could not obtain as much information from it as might be expected, but it was clear that the pine stumps were rooted shallowly in the dark *Eriophorum* layer. Digging a short way down just underneath one of the pines revealed a nest of *Menyanthes* seeds and the beginning of the *Sphagnum*-sedge peat. A sample taken and analysed from the peat among the roots of the pine was comparable to something between 150 and 160 cm. in the diagram (Text-fig. 9). This correlation, taken in conjunction with the evidence of stratigraphy and the piece of bark at 150 cm., makes it reasonable to take the portion of the diagram between 130 and 160 cm. as that corresponding to the pine stumps. From the evidence of the palstave which was lying on the roots of a pine this phase should represent a time during the Middle Bronze Age or later.

Samples were taken for pollen-analysis every 10 cm., and gave the pollen-diagram (Text-fig. 9). At the base of this diagram there is a marked maximum of alder which, from 340 to 200 cm., gives place to QM. with a maximum value of lime at 290 cm., and at 220 cm. there is a small pine maximum. Below this level the values of hazel are rather low, but upwards to the top they increase with four well-substantiated maxima. In the upper half of the diagram alder again dominates, but there is also a fair quantity of birch and QM. The notable features are: sporadic appearances of beech from 120 cm. upwards; a definite pine maximum at the level equated with that of the stumps (140-150 cm.), where the continuous lime curve ends, succeeded by an elm maximum at 90 and 100 cm., above 140 cm. the pine values are on the whole very low. The diagram will be considered further on pp. 21 ff.

BETTISFIELD

In view of the unexpectedly shallow depth of peat over such an extensive area of Whixall and Fenn's Moss, the exploration was continued in search of deeper and possibly older deposits. Consideration of the topography suggested that these might be found towards the western end of the moss. South-west of the canal the width of the moss narrows, and trial borings were made in this part.

Alder and reeds grow on the clay of which the canal bank is made, and round the south-western side of the moss is a plantation of spruce and pine, with a few trees here and there on the drier parts of the moss itself. Although there is no turf being cut here at present, the western side has been extensively cut away in recent times.



Text-fig. 9. Pollen diagram and stratigraphy at the site of the palstave from Whixall Moss. Symbols as in Text-figs. 4 and 5.

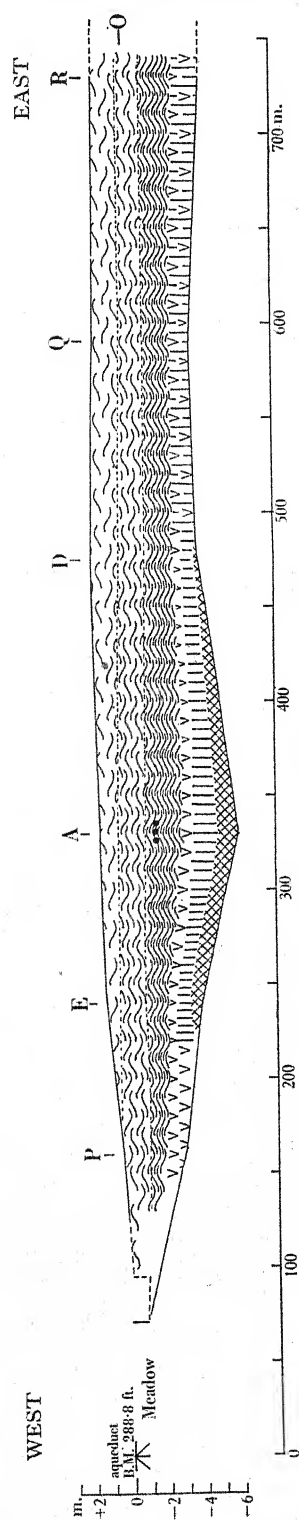
Calluna dominates the surface vegetation, with *Sphagnum*, mostly *S. papillosum*, in the ground layer.

A line of trial borings was made more or less parallel to the canal and about 60 yards from its southern bank. At the end of the driftway to the canal from Bettisfield Mill a copse extends some distance eastwards along the bank; near the end of this copse the first boring A was made, then three more eastwards and two westwards. The section, Text-fig. 10, shows the stratigraphy. The first boring A was in the deepest part of an old channel; at the bottom was a layer of gyttja mud overlain by carex peat followed by a wood peat. In borings D, Q, and R, which were shallower, the sedge and wood peat were intermingled, while towards the margin the wood peat was the primary deposit. Following the wood or sedge-wood peat is a layer of highly humified *Sphagnum* peat. At A wood was struck at 3 m., about half a metre below the top of the humified layer. The highly humified *Sphagnum* peat is succeeded by a layer with humification averaging 5-6°, but consisting of peat layers from several successive regeneration cycles. At R, where the whole series tended to be much wetter and it was difficult to find distinct boundaries between the different layers of the *Sphagnum* peat, a rhizome of *Scheuchzeria palustris* was brought up in the borer from a depth of 190 cm., which seemed to fall within the band of moderately humified peat. Above this band there was much water at all the stations east of E, but at the top there was an average of a metre of fresh yellow *Sphagnum* peat with a humification of from 1 to 3°.

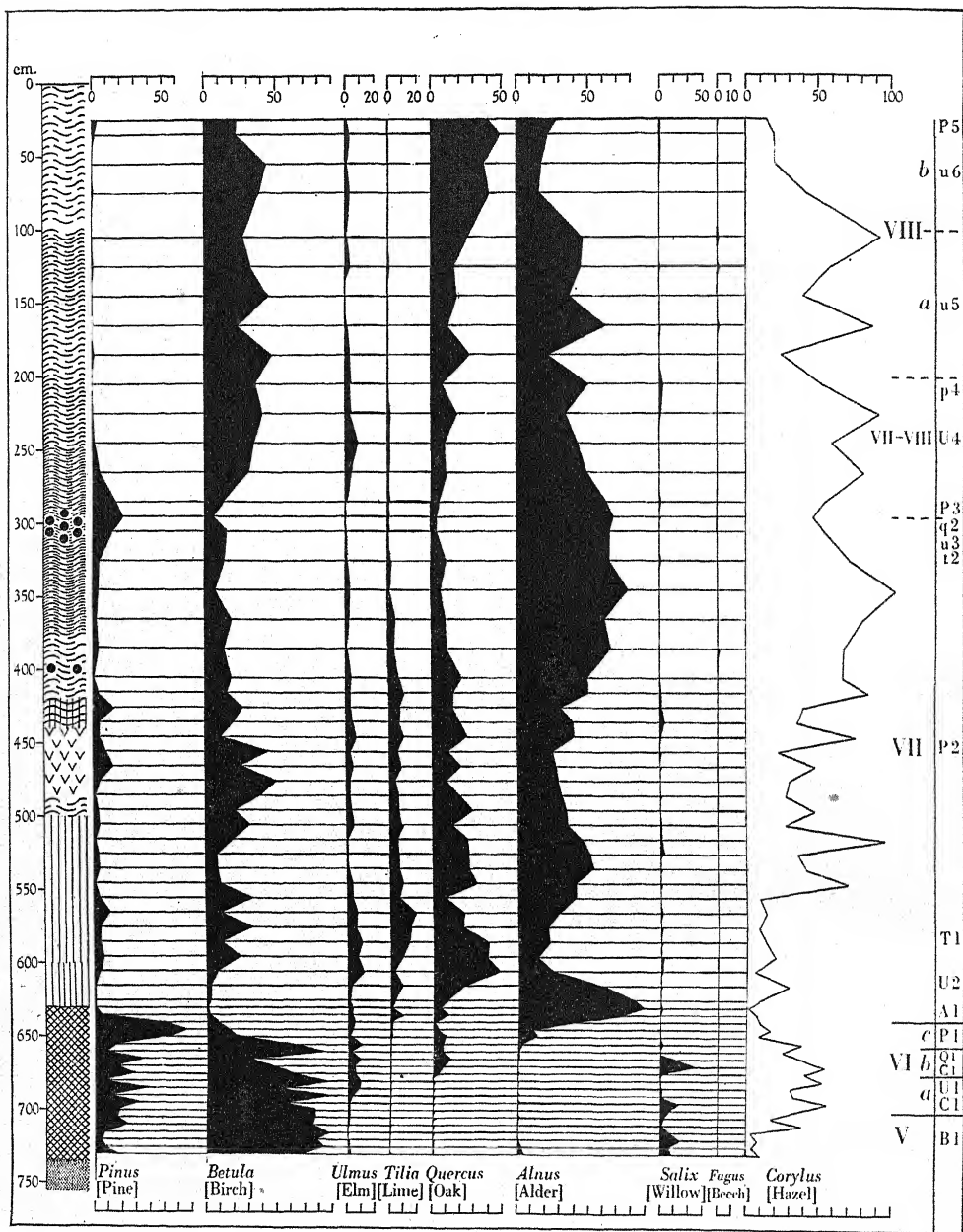
As the first boring A proved to be in the deepest part of the old channel, a series of samples was taken there for analysis, and from them the pollen diagram shown in Text-fig. 11 was constructed. Samples were analysed at every 5 cm. between 730 and 625 cm., at every 10 cm. from 625 to 405 cm., and mostly every 20 cm. above that. Between 75 and 100 cm. there was so much water that samples could not be taken.

The stratigraphy recorded was:

cm.	
0-50	Fresh yellow <i>Sphagnum papillosum</i> peat, H 1-2, <i>Eriophorum angustifolium</i> .
50-80	Fresh yellow <i>Sphagnum imbricatum</i> and <i>S. cuspidatum</i> , H 1-2, abundant <i>Eriophorum vaginatum</i> .
80-100	Fresh yellow <i>Sphagnum papillosum</i> , H 1-2, <i>Eriophorum vaginatum</i> , much water.
100-150	Light brown <i>Sphagnum</i> peat, H 6-8. Signs of regeneration complex.
150-200	<i>Sphagnum</i> , <i>Calluna</i> , <i>Oxycoccus</i> , and <i>Eriophorum vaginatum</i> , H 5-6.
200-235	<i>Sphagnum</i> , <i>Eriophorum</i> , and <i>Calluna</i> , H 7.
235-300	Black-brown <i>Sphagnum</i> , <i>Eriophorum</i> , <i>Calluna</i> . Ericaceous roots, very little structure recognizable.
295-310	Reddish bark fragment. Borer continually struck solid wood.
300-375	Dark brown <i>Sphagnum-Calluna</i> peat, <i>Oxycoccus</i> , H 7.
375-420	<i>Sphagnum</i> peat, H 3-4.
400	Red barked twig.



Text-fig. 10. Section across part of the moss south-west of the canal near Bettisfield. The bench mark is on the arch of an aqueduct passing under the canal. The underlying deposit is clay, and the section cuts across the bed of an old channel filled with organic mud overlain by sedge and then brushwood peat. Above is a considerable thickness of *Sphagnum* peat, the lower part highly humified and containing some wood, the upper part of variable humification with a very fresh layer at the top. The pollen diagram, Text-fig. 11, is from station A. Symbols as in Text-fig. 4.



Text-fig. 11. Pollen diagram from station A, Bettisfield. Symbols as in Text-figs. 4 and 5.

cm.	
410	Wood.
420-440	Transition. <i>Sphagnum</i> and <i>Phragmites</i> , H 4-5.
440-490	Birch wood, W 2-3, R 2-3.
490-500	<i>Sphagnum cymbifolium</i> , <i>Eriophorum angustifolium</i> , W 1-2, R 2-3.
500-600	Caricetum. Seeds of <i>Menyanthes</i> , fine birch twigs and bark, W 1, R 3, H 3.
600-630	<i>Phragmites</i> , abundant seeds of <i>Menyanthes</i> , birch wood.
630	Sharp contact.
630-650	Dark brown to black mud, occasional fragments of bark, R 1, H 7-8.
650-740	Gyttja, seeds of <i>Menyanthes</i> , some yellow wood fragments.
740-745	Black clay.
745-750	Stiff grey clay.
750-	Pink clay.

The base of the diagram, from 730 to 655 cm., has a dominance of birch; pine is variable in inverse ratio to the oscillations of the birch curve. Below 710 cm., hazel is very low; and QM. is represented by only 1-4% below 695 cm. 640-650 cm. is marked by a striking dominance of pine, rapidly succeeded by alder and then oak. From 555 to 425 cm. QM. alder, and birch are all important, and between 465 and 425 cm. there is a small increase in pine. From 425 to 245 cm. alder is the strong dominant; but a decided pine maximum culminates at 295 cm., corresponding to the level at which wood was encountered in the boring. It should be noted that the lime curve ceases to be continuous above this point. The top of the diagram is characterized by rising birch and oak, considerable maxima of hazel, and very low and sporadic values of elm, lime and pine. Beech appears at 165 and 125 cm.

GENERAL DISCUSSION AND COMPARISONS

Before attempting an interpretation and discussion of the results of the investigations, some facts relating to the stratigraphy which the borings at these three sites have revealed may be summarized here.

At Whittall there had been a mere with very deep water close under the south-west bank. Over a bed of clay and gravel accumulations of organic mud had formed continuously for a great depth, interrupted only by a band of detritus mud near the bottom. These mere deposits were sealed by a layer of fresh *Sphagnum* peat some 4 ft. thick.

At Whixall and Bettisfield stiff pink clay underlay the moss, and was overlain by gyttja mud with wood fragments, then a considerable thickness of sedge peat followed by a layer of cotton-grass and highly humified *Sphagnum* peat with pine stumps *in situ*. Above, the highly humified peat continued for a short way, gradually becoming less humified but in varying degrees. Fresh *Sphagnum* peat 60-100 cm. thick formed the surface layer.

In Fenn's Moss fairly numerous remains of the stems and rhizomes of *Scheuchzeria palustris* (marsh *Scheuchzeria*) embedded in *Sphagnum cuspidatum* were observed in

patches near the base of the upper undecayed *Sphagnum* layer, and one rhizome was found 190 cm. below the surface at station R at Bettisfield. This species was recorded growing on Welshampton Moss, the borders of Ellesmere Mere, and at Bomere Pool in 1908 (*Victoria County History*, 1908, p. 61). It is rare in England, and has generally a more arctic distribution. In Jutland, Prof. Jessen (Jessen, 1935) has found it at the base of the "Sub-Atlantic" peat immediately above the "gräns"¹ (Jessen, 1935, Fig. 2). It is a plant which grows half-submerged on the borders of pools on the bog surface, and with the great abundance of *Sphagnum tenellum* at the same levels may be considered indicative of very wet conditions on the bog surface. This is also the case in Denmark where *Scheuchzeria* is abundant in the early Sub-Atlantic layers though very rare as a living plant to-day. Other evidence for the diminution or disappearance of raised-bog species which were abundant in the Sub-Atlantic has been given by Godwin & Conway (1939).

On consideration of the pollen diagrams from these three sites certain characteristic features were detected in them sufficiently clearly to warrant a division into a number of phases. These phases or zones have been essentially dictated by a study of the diagrams and stratigraphy in this area, and are intended to have validity for this only. At the same time there has been taken into account the zoning proposed by Godwin for the East Anglian Fenland (Godwin, 1939), which has been used by the Claphams for Berkshire (Clapham, A. R. & B. N. 1939), the Tregaron bog zones from Wales (Godwin & Mitchell, 1938), and particularly the zones used by Jessen for his intensive studies in Denmark, and those he has tentatively proposed for Ireland.

The sequence from Whattall (Text-fig. 5) appears to be the oldest, reaching back to a period before alder pollen was represented; and all the earlier phases are more vividly defined in this diagram than in the others. Bettisfield (Text-fig. 11) seems to begin slightly later, and Whixall (Text-fig. 9) not until alder has become a dominant species. The later phases are more distinctly visible in the Bettisfield and Whixall diagrams, partly on account of the local influences which dominated Whattall when it was overgrown by *Sphagnum* and birch.

After comparison with Prof. Jessen's schemes for Ireland (Jessen, 1938; Mahr, 1937) and Denmark (Jessen, 1935), and taking into consideration some corroborative evidence from Cornwall, it was decided to assume that his phases I, II, and III for the Late Glacial Period might be expected in this country too, but are absent from these diagrams.² Jessen's phase IV (Ireland) is described as "Birch dominating, much willow", and the hazel curve does not start until the next phase. The lower band of gyttja at Whattall contained a great dominance of birch (B 1)³ and virtually no QM. or hazel. It was decided, therefore, in spite of the relative scarcity of willow,

¹ In this instance, the Sub-Boreal-Sub-Atlantic gräns (Granlund's (1932) RY III) between Jessen's phases VIII and IX. See p. 391.

² His phase III is frequently characterized by a more or less sterile solifluction layer, which could possibly be represented by the underlying pebbly clay at Whattall, through which the peat auger could not penetrate.

³ The letters and figures in brackets refer to maxima and minima of pollen seen in the diagrams and set out in Table I. Capital letters indicate maxima and small letters minima.

to call the part between 520 and 500 cm. zone IV and align it with Jessen's IV. This phase is not represented in the other diagrams.

In the next phase, V, hazel begins and increases, there is a maximum of willow, and feeble but practically continuous curves of oak and elm with occasional lime, the total QM. never exceeding 10 %. Alder appears, but rarely as much as 5 %, and neither it nor QM. have any tendency to rise towards the top. At Whattall this phase extends from 500 to 440 cm., and at Bettisfield from 730 to 695 cm. A more expanded portion which must be put somewhere at the beginning of this phase is seen in the diagram (Text-fig. 6) from the 50 cm. of detritus gyttja, 1300-1350 cm., taken at station A at Whattall. Here it will be noticed that there is an overwhelming dominance of birch with but little pine and willow, QM. and alder appear here and there, and hazel which begins weakly expands towards the top.

Continuing upwards we have numbered the next phase as VI, and not Vb as Jessen does, believing that the startlingly sudden development of hazel and QM. with corresponding fall in birch from its absolute dominance in IV and V is worthy of greater differentiation than between an (a) and a (b) phase. This phase VI begins at Whattall at 440 cm., where the coarse detritus gyttja is superseded by a purely organic nekron mud. The most characteristic feature at Whattall is the enormous rise of hazel which between 430 and 440 cm. moves from 80 to 596 %. At Bettisfield there is also a rise in hazel, but it does not reach more than 55 %; and it is difficult to understand this discrepancy between the two sites as they are only about 4 miles apart. The upper limit of VI is drawn at the point where alder first expands noticeably, and where its rising curve crosses the falling pine and birch curves. Within this period dominated all through by the hazel maximum (C 1) there are some well-marked subsidiary phases which may also be seen in many diagrams from other places although they attracted little attention. They were recognized by Godwin & Mitchell in zones E 1, E 2, and E 3 at Tregaron, Wales—and they form part of Godwin's zones for East Anglia. Here they display themselves so plainly that considerable emphasis must be laid on them.

(a) There is a marked maximum of elm (U 1), at Whattall reaching 38 % at 420 cm., while the other trees show little change from V.

(b) The elm maximum gives place to one of oak (Q 1).

(c) Pine, which at Whattall has been rather low but gradually rising, and at Bettisfield has alternated with birch, now reaches a maximum (P 1), in this instance more striking at Bettisfield than at Whattall. In the former diagram, between 655 and 650 cm., it rises from 15 to 56 %. At the end of the phase it falls away as steeply as the alder rises.

Approaching the later periods it becomes more difficult to find any constant or fixed delimiting points. Phase VII begins at the base with the alder maximum (A 1), soon followed by a rise in elm (U 2), and the greatest maximum of lime (T 1). Hazel, birch, and pine have retreated, QM. and alder dominate. We have taken as the end of this phase the stage marked in the stratigraphy at Whixall and Bettisfield by pine stumps, and in the pollen diagrams by a maximum of pine (P 3) accompanied by minima of elm (u 3) and oak (q 2); with at the same time, or shortly after, the

termination of the continuous lime curve (t 2). The latter feature is one more constantly met with than the others in almost any diagram where lime is represented. The pine maximum (P 3) is not always present, largely because it is a local effect which may sometimes appear in the form of a birch maximum. At Bettisfield the pine maximum culminates clearly about 295 cm., and at Whixall at 140-150 cm., just above the end of the lime curve. At Whattall, however, lime has appeared more strongly all through, and maintains itself until it falls away with the pine curve above 220 cm.

In the upper part the diagrams assume a different aspect, there are sporadic appearances of beech, hornbeam, and spruce; pine and lime retreat altogether or to only 1 or 2 %; birch and oak have a tendency to rise; alder and hazel are very variable, especially the latter which swings almost rhythmically from maxima to minima. The most constant features seem to be a maximum of elm (U 4) near the base, coinciding with the disappearance or minimum (p 4) of pine, and succeeded by a minimum (u 5) visible in all three diagrams. This point corresponds approximately with, or is just above the level of a recurrence surface or gräns seen in the stratigraphy. There is not sufficiently conclusive data in these diagrams to decide definitely whether the end of phase VII should be drawn at the level of the pine stumps or at the level of this recurrence surface. The pine stumps mark a stage which can be seen both in the stratigraphy and in the pollen diagram; but, on the other hand, the continuance above them of highly humified peat may indicate that they are only one dry or drier phase in a period which should be treated as a whole up to the point at which the recurrence surface marks a real change in the climatic conditions. This indeterminate portion between the stumps and the recurrence surface we have, therefore, called VII-VIII, until further evidence of its true nature is forthcoming.

The succeeding phase, VIII, is in the diagrams hardly distinguishable in character from VII-VIII; birch, oak or alder may dominate, elm and pine are low, lime is virtually absent, and hazel very variable. VIII (a) and (b) are separated by the stratigraphical recurrence surface found at Bettisfield. In (a) there is a minimum of elm (u 5): in (b) there is another elm minimum (u 6), and a small maximum of pine (P 5) near the top.

The table (Table I) of the phases and their characteristics shows on the right side the distinctive features of maxima and minima or of the stratigraphy on which the divisions have been based and one diagram synchronized with another.

Correlation of the phases suggested here with the standard classification put forward by Blytt and Sernander is as follows:

Pre-boreal	Phase IV
Beginning of the Boreal			Phase V
Full Boreal	Phase VI
Atlantic and Sub-Boreal			Phases VII and VII-VIII (the stage P 3 would be taken to represent the Sub-Boreal)
Sub-Atlantic	Phase VIII (a) and (b)

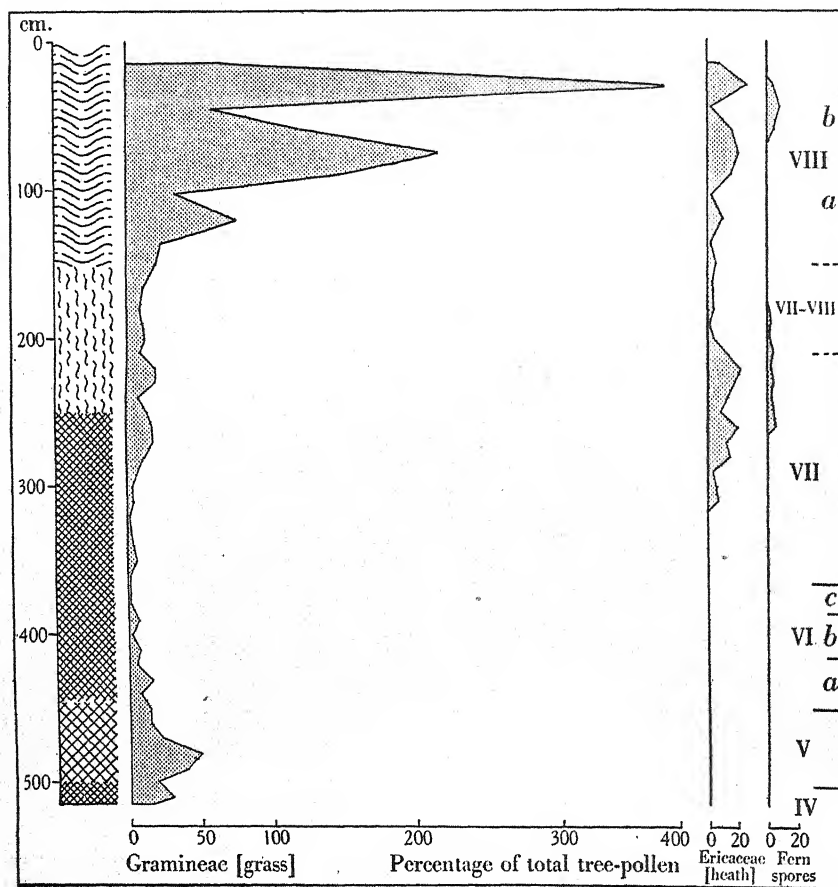
Table I. The zones of the pollen diagrams and their characteristics. On the left side are the numbers of the zones, and on the right are the letters and figures referring to the distinctive features of maxima and minima of pollen. Capital letters indicate maxima and small letters minima

A = *Alnus* (alder), B = *Betula* (birch), C = *Corylus* (hazel), P = *Pinus* (pine), Q = *Quercus* (oak), T = *Tilia* (lime), U = *Ulmus* (elm)

VIII	b	<i>Betula</i> , <i>Quercus</i> , <i>Alnus</i> may all be dominants according to locality. <i>Pinus</i> as in (VIIIa), with small max. at top. Almost no <i>Tilia</i> . Low <i>Ulmus</i> persisting, with min. near top. Sporadic <i>Fagus</i> , <i>Carpinus</i> , and <i>Picea</i> .	P ₅ u ₆
		Stratigraphical recurrence surface—"Gräns".	
	a	<i>Ulmus</i> min. <i>Tilia</i> absent, or present in very low values. <i>Quercus</i> , <i>Alnus</i> , <i>Corylus</i> very variable.	u ₅
VII-VIII		Stratigraphical recurrence-surface—"Gräns".	
		Consistent <i>Ulmus</i> max. Beginning of sporadic <i>Fagus</i> and <i>Carpinus</i> or <i>Picea</i> . Continuous <i>Pinus</i> and <i>Tilia</i> curves end at top. <i>Betula</i> may rise. <i>Quercus</i> , <i>Alnus</i> , <i>Corylus</i> variable but important.	P ₄ U ₄
VII		<i>Pinus</i> max., stumps. <i>Tilia</i> curve petering out. Consistent <i>Ulmus</i> , <i>Quercus</i> min.	P ₃ u ₃ q ₂ t ₂
		Close to base <i>Alnus</i> often very high, with greatest <i>Tilia</i> max. following a secondary <i>Ulmus</i> max. <i>Pinus</i> low with one max. in middle and one at end. <i>Betula</i> , QM., <i>Alnus</i> in fair amount. Local dry horizon— <i>Pinus</i> bark.	P ₂ T ₁ U ₂ A ₁
		<i>Pinus</i> , <i>Betula</i> fall. <i>Tilia</i> , <i>Alnus</i> rise, latter often very steeply.	
VI	c	<i>Pinus</i> max. <i>Betula</i> , <i>Ulmus</i> , <i>Quercus</i> as before. <i>Alnus</i> and <i>Tilia</i> rising at top. <i>Corylus</i> falls.	P ₁
	b	<i>Ulmus</i> falls from (a) giving place to <i>Quercus</i> max. <i>Betula</i> rather low. <i>Alnus</i> may begin to rise. <i>Corylus</i> still very high. <i>Pinus</i> may rise. <i>Tilia</i> , <i>Salix</i> as in (a).	Q ₁ C ₁
	a	Rapid fall in <i>Betula</i> , <i>Ulmus</i> rises to highest max. <i>Corylus</i> very high max. <i>Quercus</i> rising towards (b). <i>Pinus</i> , <i>Tilia</i> , <i>Alnus</i> , <i>Salix</i> low.	U ₁ C ₁
V		<i>Betula</i> dominating, little <i>Pinus</i> . First appearance at base of low values of <i>Ulmus</i> , <i>Tilia</i> , <i>Quercus</i> , <i>Alnus</i> , sum about 10%. <i>Salix</i> max. in middle. <i>Corylus</i> begins at base, and keeps rather low values but increasing throughout, especially at the top.	B ₁
IV		<i>Betula</i> dominating, little <i>Pinus</i> and <i>Salix</i> . QM. and <i>Corylus</i> almost completely absent.	B ₁
III II I		} Late Glacial.	

As indicated above (p. 384), phases I-IV are based on Jessen's scheme for Ireland, the later phases may be correlated roughly as follows:

Jessen Va	Phase V
Jessen Vb	Phase VI (a), (b), and (c)
Jessen VI	Phase VII and possibly VII-VIII
Jessen VII and VIII	Phase VIII and possibly VII-VIII



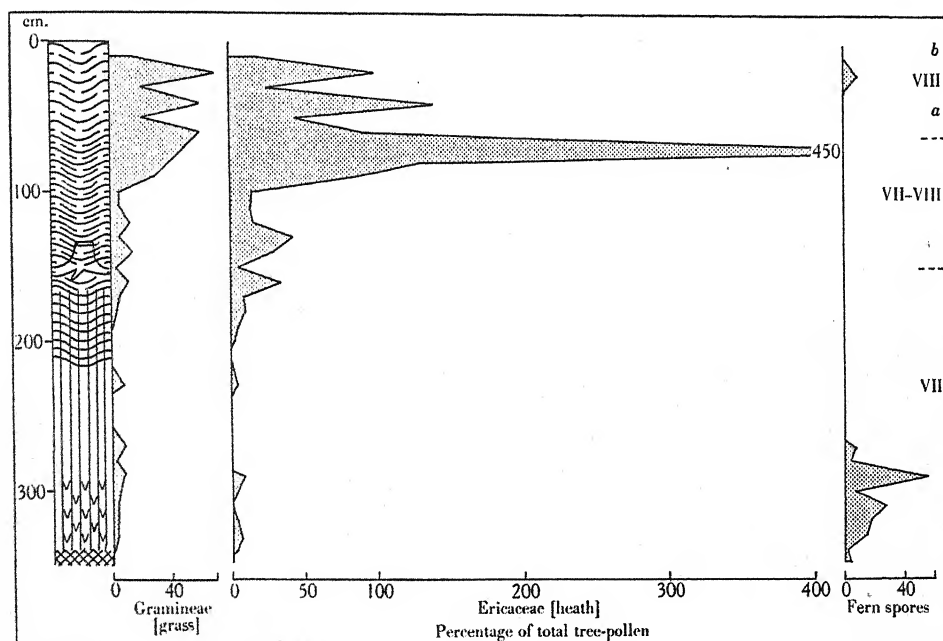
Text-fig. 12. Whattall Moss, station 7. Select species of non-tree pollen reckoned as percentage of the total tree pollen shown in Text-fig. 5. Symbols as in Text-figs. 4 and 5.

Exactly what constitutes the division between Jessen's VII and VIII is not stated in the preliminary account given in Prof. Mahr's recent review of Irish Prehistory (Mahr, 1937).

The diagrams (Text-figs. 12-14) of select species of the non-tree pollen for the most part bear out the themes of the tree pollen curves. It will be noticed that in phases IV and V at Whattall (Text-fig. 12), although the values are low, there is a maximum of grass as compared with a decided minimum in period VI when

forest-forming trees seem to have taken the place of birch scrub or open country. At Whattall, however, the non-tree pollen plays but small part until the formation of the *Sphagnum* peat when there is a marked increase of grass pollen. One feature which supports the evidence of the tree pollen and stratigraphy is the maximum of ericaceous pollen between 295 and 220 cm. at the stage which appears to have been dry and to have been succeeded by a wetter period (that to which the canoe belongs) during which the ericaceous pollen declines again.

At Bettisfield (Text-fig. 14) there is a steep maximum of ericaceous pollen at 405 cm. where, on other grounds, we have supposed (see p. 391) that conditions were dry; a similar feature can be seen at 295 cm., the level of the pine wood.

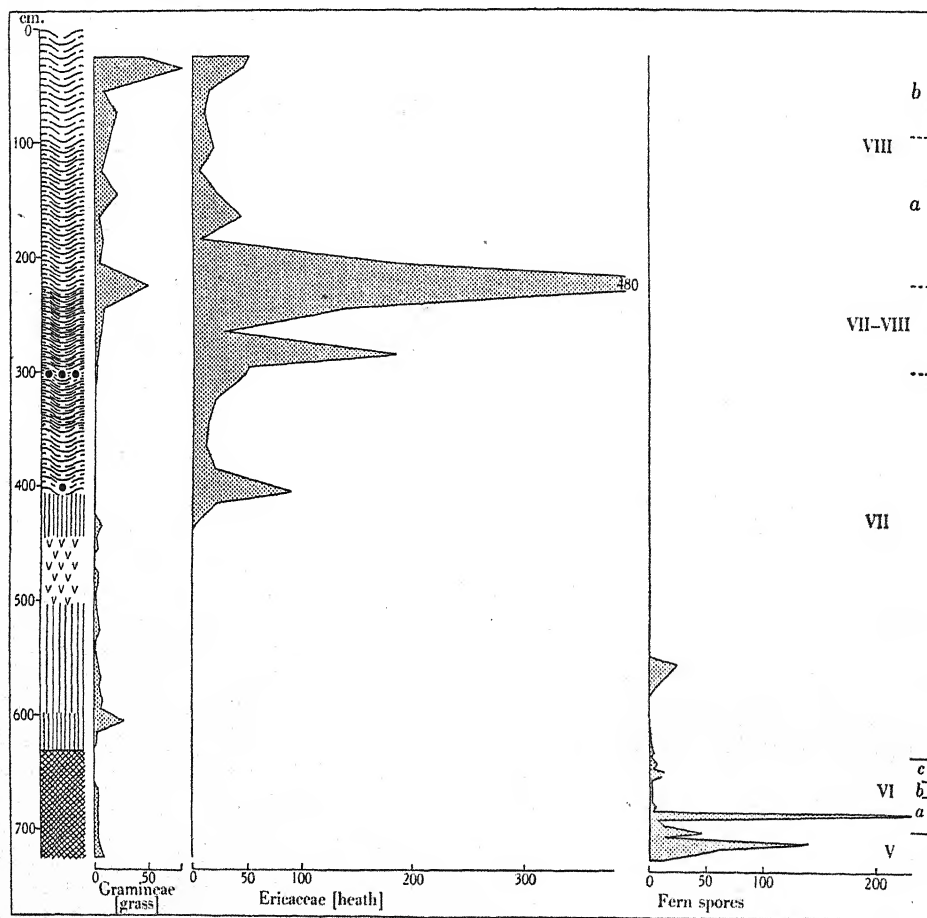


Text-fig. 13. Whixall Moss, palstave site. Select species of non-tree pollen reckoned as percentage of total tree pollen shown in Text-fig. 9. Symbols as in Text-figs. 4 and 5.

At Whixall (Text-fig. 13) the increase of ericaceous pollen at the level of the pine stumps is very small. The most helpful feature of these two diagrams is the enormous maximum reached by ericaceous pollen at 225 and 70 cm. at Bettisfield and Whixall respectively, where the stratigraphy affords only rather meagre evidence of the gräns presumed to be equivalent to RY III of Granlund. The presence of such high values of ericaceous pollen must surely imply a drying of the surface of the moss; and the rapid and complete fall which succeeds the maximum indicates the advance of wetter conditions.

The general story of the forest history shown in these diagrams accords with Prof. von Post's idea (von Post, 1930) of the post-glacial development in which conditions ameliorated until they reached a climatic optimum succeeded by deteriora-

tion and reversion towards the previous pessimum. In the Shropshire series the amelioration can be seen in the gradual replacement of birch by QM. during phases V and VI, and the optimum appears in the central phase VII where a warmth-loving species such as lime reaches its greatest development. The tendency towards reversion may perhaps be seen in the decline of lime, and the increase of birch and oak (possibly *Quercus sessiliflora*) in phases VII-VIII and VIII. Certainly,



Text-fig. 14. Bettisfield, station A. Select species of non-tree pollen reckoned as percentage of total tree pollen shown in Text-fig. 11. Symbols as in Text-figs. 4 and 5.

taken as a whole, the birch curve is distinctly concave, while those of alder and lime are convex.

The behaviour of hazel in the later periods is at present inexplicable; its oscillations are generally well-substantiated as though they had some definite meaning, but one cannot rely too implicitly on the values in the *Sphagnum* strata where there is a possibility of confusion of its pollen with that of *Myrica gale* (sweet gale) which frequently grows on the surface of a *Sphagnum* bog. Typical grains of *Myrica* and

hazel are easily distinguishable, but there is a greater number of grains about which one cannot be other than dubious even though one's opinion inclines to one species rather than the other.

It has been customary since the pioneer work of Blytt and Sernander to adopt their division of the post-glacial period into the climatic phases Pre-boreal, Atlantic, Sub-Boreal, and Sub-Atlantic, using them also to indicate periods of time. While the retention of these terms to denote the type of climate prevailing at different times is convenient, a too rigid use is not desirable, since the increasing volume of evidence shows more and more plainly that the story is in a way simpler, and in other respects more complex than this fivefold division suggests. Taking von Post's threefold scheme of amelioration, optimum, and deterioration as a basis, Granlund's (Granlund, 1932) work in Sweden has demonstrated the possibility of superimposing upon this groundwork a number of smaller climatic cycles. He has discovered and dated in the raised-bogs of south and central Sweden five so-called "recurrence levels" (RY), each marking a stage at which the moss has again started to grow luxuriantly after a period of retardation probably due to dryness. Of these five, RY III, the junction between Sub-Boreal and Sub-Atlantic times, has generally been considered the most important one, and that which could be seen so strikingly in many raised-bogs of north-western Europe. Similarly, in the bogs of Norwegian coastal districts Ording (Ording, 1934) has found evidence of three dry phases since the transition between the Sub-Boreal and Sub-Atlantic.

In the Shropshire series too there are signs of comparable phenomena. Starting from the base, the first indication of a dry phase is VI (c),¹ in which pine reaches such a notable maximum (P 1) at Bettisfield, and it appears quite clearly at Whattall also, but the Whixall series does not reach back to this stage.

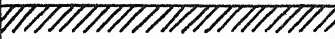

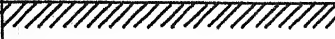
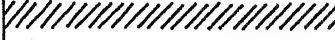

The next evidence for drier conditions occurs during phase VII, when there are small but definite pine and birch maxima at Bettisfield from 475 to 425 cm. (P 2), at Whixall from 230 to 220 cm., and at Whattall from 290 to 280 cm. It is corroborated in the stratigraphy at Bettisfield by the presence of a band of *Eriophorum vaginatum* and a piece of pine bark. This stage, however, is not so forcibly expressed as the next, to which the pine stumps and highly humified peat at Whixall and Bettisfield bear ample testimony. In the diagrams from these sites there is a sharp pine maximum (P 3) at 150 and 295 cm. respectively. At Whattall pine has remained fairly high, but it has a secondary maximum from 230 to 220 cm. where, on the stratigraphical evidence, the growth of moss in the gyttja suggests that the water had become shallower. Two further indications of dry conditions in the later periods are afforded by the recurrence surface (Gr 1) between phases VII-VIII and VIII, and that (Gr 2) between phases VIII (a) and VIII (b).

It is not proposed to give any names to these five stages other than P 1, P 2, P 3, Gr 1, and Gr 2, for one would not be justified in classing them as recurrence levels of general importance until they are supported by evidence from elsewhere. At the same time one must recognize that in this locality, at least, there was a succession

¹ This cannot be correlated with any of Granlund's RY, as his are all subsequent to the expansion of alder.

of wetter and drier periods in which the contrasts between one and the other were sufficient to leave their mark on the floral history of the region. The table (Text-fig. 15) shows the occurrence and evidence for the five drier stages.

One may attempt to link these stages to local cultural history through archaeological finds. There is good evidence that the Middle Bronze Age palstave was associated with the pine stump layer at Whixall, as it was lying on the roots of one of the stumps. Possibly it reached that position some time after the tree had been growing there, but it is unlikely that the tree would be very much earlier than the

Sub-Atlantic	VIII	<i>b</i>			
		<i>a</i>		Recurrence surface (gräns)	Gr 2
Atlantic	VII-VIII			Recurrence surface (gräns)	Gr 1
	VII			Pine stumps and pollen. High humification	P 3
				Pine bark and pollen Beginning of raised-bog growth	P 2
Boreal		<i>c</i>		Pine pollen maximum	P 1
	VI	<i>b</i>			

Text-fig. 15. Occurrence and evidence of "dry" phases indicated by cross hatching. The figures on the left refer to the zones in the pollen diagrams, and the symbols on the right to the three maxima of pine and to the two recurrence surfaces (gräns) observed in the stratigraphy.

palstave. In fact, accepting the evidence offered, the tree layer should be dated to the Middle Bronze Age or slightly later; making the end of phase VII, or phase VII-VIII belong to Middle and Later Bronze Age times, perhaps between 1200 and 800 B.C. There is no direct evidence for dating P 1, P 2, or any other stage of the forest history.

We have observed (p. 373) that the canoe at Whattall probably lay at a depth between 150 and 190 cm. from the surface, corresponding in the diagram to the time of the elm maximum (U 4) after the opening of phase VII-VIII, and above the pine maximum (P 3) which is associated with the stumps at Whixall. The canoe must, therefore, be assigned to phase VII-VIII times, and is thus definitely *later* than the

palstave. Although we cannot yet fix a lower limit for her date, her position in relation to the Bronze Age level, and below that possibly equivalent to the recurrence surface of RY III, suggests that she most likely belonged to the end of the Late Bronze Age or Early Iron Age. In relation to the local fluctuations of climate it appears that the Middle Bronze Age was a dry period, while the period to which the canoe belonged was wet, or at least wetter.

At these three sites the generally expected recurrence surface (RY III) is not striking. This may be due to the character of the sites which was perhaps such that the climatic changes at that time made but little impression, or it may be that this part of the country was not greatly affected by that particular climatic disturbance. Brooks (Brooks, 1927) has put forward the suggestion that conditions suitable for producing a recurrence surface would not operate at the same time over the whole area from Ireland to Scandinavia, or even from one side of England to the other; but that excessive precipitation in one region would induce normal or abnormally dry conditions in the other. Granlund has shown that there is more than one recurrence surface; and if Brooks's interesting theories can be further substantiated and elaborated, the cataclysm hitherto supposed to have produced one and the same recurrence surface over the whole of north-western Europe may be found to have been more limited in its sphere of influence.

In Shropshire, especially at Bettisfield, the evidence of the later periods can most simply be interpreted as a succession of dry stages during phase VII culminating at P 3, succeeded by increasingly damper conditions when the pines were killed and overgrown by *Sphagnum*; and although at first a high degree of humification was maintained, it seems gradually to have decreased during a period of considerable instability producing variable but on the whole decreasing humification,¹ until at 100 cm. there is a clear recurrence layer although to what age this belongs we have as yet no evidence to show.²

Further exploitation of the available evidence from these sites and from the local history does not seem possible at present, but there is some valuable comparative material from other regions.

The nearest site at which pollen-analytical investigations of a raised-bog have been made is Chat Moss, 9 miles west of Manchester, and 25 m. above O.D. Lektor Erdtman has published an account of it (Erdtman, 1928). The diagram composed by him (Erdtman, 1928, Fig. 6) shows some similarity to that from Whattall. The lowest samples at Chat Moss may be equivalent to our phase V; and stages VI (a), (b), and (c) are quite clear although the pine phase (c) begins during the oak maximum of (b), and is thus more protracted. In the upper layers both lime and pine are very poorly represented, and the diagram is disappointingly characterless, although lime does seem to disappear just after one of the feeble appearances of pine and is followed by an elm maximum similar to U 4 in phase VII-VIII. The

¹ These effects may be due to the interplay of temperature and humidity in controlling the degree of humification.

² Its position in relation to the depth of the stumps and to the surface forces one to suppose that it may be mediaeval, otherwise the rate of formation of the peat before and after it must have been so extraordinarily different.

recurrence surface recorded by Erdtman occurs at this point, in a position similar to the lower one (Gr 1) at Bettisfield. There is nothing at Chat Moss to contradict the deductions made from the Shropshire material, and it does to some extent substantiate them.

To the west, a comparable site in Wales is the large raised-bog lying at about 160 m. o.d. near Tregaron, Cardiganshire (Godwin & Mitchell, 1938). Here there is a clearly marked recurrence surface in the stratigraphy, with a "retardation" layer above it. The earlier phases of the pollen diagrams correspond well with the Shropshire results. Stages VI (a), (b), and (c) are well defined in the diagram from W 27 (Godwin & Mitchell, 1938, Fig. 5). As at Chat Moss the later periods lack distinctive features, and there is very little of the pine and lime which are so helpful in Shropshire. Such lime as there is, however, occurs mostly in the part between the expansion of alder and the recurrence surface; and in two of the diagrams (W 27 and SE 6) there are signs of an elm maximum (?U 4) following it. The hazel curves fluctuate strikingly as in the Shropshire diagrams. (See also postscript.)

Dr Godwin (Godwin & Clifford, 1938) has published a number of diagrams from the East Anglian Fens which may be compared with those from Shropshire. In the series through the lower peat bed at Peacock's Farm (Godwin & Clifford, 1938, Fig. 36) phases corresponding to VI (a), (b), and (c) can clearly be seen although the overwhelming dominance of pine until it is replaced by the expansion of alder prevents phase (c) from being distinctly demarcated. In the Fenland changes in the relative levels of land and sea have caused swamping and drying of the land, and consequent variations in the vegetation appearing in the diagrams as possibly "dry" and "wet" zones which may in no way be related to changes in climate and precipitation. Although, therefore, possibly "dry" phases may be seen in the diagrams from Woodwalton (Godwin & Clifford, 1938, Figs. 14, 15), Trundle Mere (Godwin & Clifford, 1938, Fig. 17), and Wilton Bridge (Godwin & Clifford, 1938, Fig. 41), they cannot necessarily be equated with P 2 or P 3 in Shropshire. At the same time it may be significant that at all these sites there is a maximum of pine similar to P 3 when the main representation of lime ceases and beech develops.

Turning to the continental evidence, some comparable results are to be found in Prof. Jessen's work on north Jutland (Jessen, 1935). The earlier post-glacial phases in Scandinavia are not exactly parallel to those farther west, as in the former region the expansion of alder, oak, elm, and lime was practically contemporaneous. But it is interesting to note that lime generally disappears where beech comes in at the Sub-Boreal-Sub-Atlantic recurrence surface (RY III), between Jessen's strata VIII and IX, and above which there is at Brøndum (Jessen, 1935, fig. 3) a layer of *Scheuchzeria* peat.

Near Bremervörde, on the Oste in north-west Germany (Schubert, 1933), a bronze palstave was found in a Moss which was examined pollen-analytically. The palstave is of a type belonging to period II (Montelius) of the Northern Bronze Age, and dated to 1600-1400 B.C. (or to 1350-1250 according to other opinions (Åberg, 1935)). The level of the find was at the point in the diagram where lime virtually goes out and beech begins. The implement lay in highly humified *Sphagnum*

peat 35 cm. below the Grenz. This find is therefore almost identically similar to that of the palstave from Whixall; but it should be remembered that beech appeared somewhat earlier in north-western Germany than in Scandinavia, and its curve usually begins below the recurrence surface of RY III.

Although it would be imprudent and premature to draw decisive conclusions from such limited data as the Shropshire material affords, there are a few features which appear to be the most significant, and to which I should like to draw attention without insisting on their intrinsic importance:

- (1) The recognition of phases (a), (b), and (c) in period VI, the full Boreal.
- (2) The lime maximum (T 1) early in period VII.
- (3) The stage here called P 3 which *may* have been the driest phase, and which usually marks the end of the constant lime curve and the first appearance of beech.
- (4) The elm maximum (U 4) succeeding the stage P 3.
- (5) The lack of a clear recurrence surface assignable to RY III, combined with the possibility of several "dry" phases or "recurrence surfaces", P 1, P 2, P 3, Gr 1, and Gr 2.

How far the scheme outlined for the Shropshire region may be applicable to other parts of Britain is not yet apparent, as until further data is available from which to reconstruct the history of the post-glacial forests and climate of this country, any scheme of interpretations founded on such local and limited observations cannot pretend to be more than a tentative plan which further information will amplify or refute. So far as possible, therefore, the facts which the work has brought to light have been kept apart from deductions evolved partly in imagination.¹

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¹ Since writing this paper an article has been published (Hyde, H. A. (1939). On the date of an axe-hammer from Llangetho, Cardiganshire. *Proc. Prehist. Soc.* V, 166-172.) showing that a perforated stone axe-hammer thought to belong to the Late Bronze Age was found in a bog near Tregaron at a level probably slightly later than that of the Whixall palstave.

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EXPLANATION OF PLATE IV

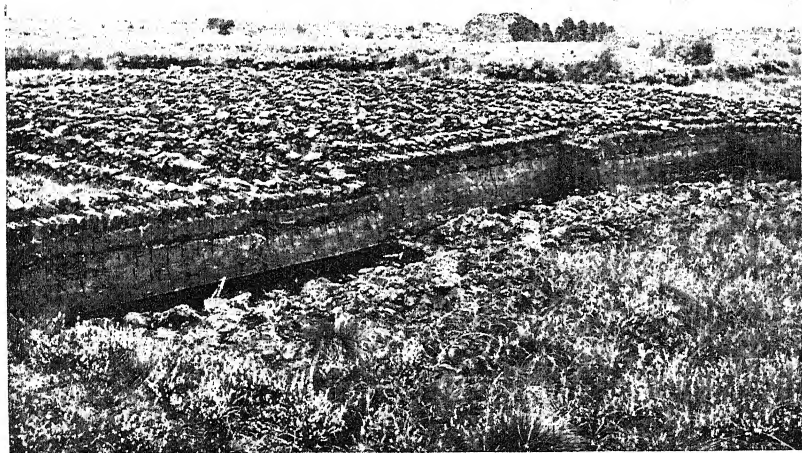
(a) Crose Mere, looking east. The ridge jutting into the trees in the middle distance separates the waters of the mere from Whattall Moss. Crossing the foreground is the main road from Ellesmere to Shrewsbury. This photograph shows the character of the country around Whattall.

(b) Whixall Moss. Face of peat working. Cut turves spread out for drying, and peat stacks in the background. Note the typical surface of the drained moss and its immense size. The dark band across the face of the working is probably a layer of *Eriophorum*.

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(a)



(b)

REVIEWS

An Introduction to Botany. By ARTHUR W. HAUPT. 9×6 in. Pp. 396. 278 figures. London: McGraw-Hill Publishing Co. Ltd., 1938. Price 18s.

There are two aspects of Botany which, in the reviewer's opinion, should be clearly indicated in any teaching course. The first is the "historical" aspect, which includes comparative morphology, heredity, variation and evolution. The second is the "physiological" aspect, and it concerns the relations between plant form and function and between the plant and its environment; it includes ecology and physiology.

The "historical" aspect commonly receives much more attention than the "physiological". Probably the best way of treating the latter is to study the flowering plant, say, to interpret respiration, assimilation, conduction, growth and so on in terms of the cell and its metabolism, and then to relate the different functions to the different kinds of cell in the plant. In this way, investigation of plant structure may go hand in hand with a series of simple physiological experiments. The ecological side may be dealt with in a very broad way, stressing perhaps the correlation between climate and type of vegetation.

Mr Haupt's book begins with the morphology of the flowering plant, and the physiology is left to take care of itself. For example, we read, on pp. 34 *et seq.*, of sessile leaves, stipules, net-veined leaves, pinnate leaves, crenate and linear leaves, leaf-fall, evergreen leaves, scale-leaves, tendrils, thorns, and the leaves of the pitcher-plant; and on p. 67 we have an account of leaf tissue. We have to wait until p. 108 for photosynthesis. Respiration arrives on p. 115.

Mr Haupt states in his preface that he believes this is the best way of doing things; and in venturing to disagree, we do not wish to overstress what we think are the faults of his book, which in many ways, especially as regards the "historical" aspect, is very good. There are excellent chapters on Flowers and Fruit, Floral Evolution and Pollination, Heredity, and Plant Life of the Past; and there is a good review of the lower plants which illustrates the evolutionary principle.

On the whole, however, not only is the structure-function relation neglected, but the physiology which is given, is inadequate; two chapters out of twenty are devoted to it, and they are miserable stuff. Practically no attempt is made to show that there is an experimental side to the subject. The biochemical treatment is also very poor; in the index, there is no reference to starch, and only one to cellulose, and both receive scant attention in the text.

The book is designed for use during the first half of a year's course in general Biology; the second half would be occupied by Zoology. Its standard is about that of an English Higher School Certificate. It is well printed and bound, and very well illustrated by original photographs and drawings.

D. H. VALENTINE.

Heredity, Eugenics and Social Progress. By H. C. BIBBY. 7×4½ in. Pp. 91. 28 figures. London: Victor Gollancz, 1939. Price 1s. 6d.

This booklet is designed for popular consumption; it is No. 25 of the series "The New People's Library". The aim of the series is that each book should be authoritative, simply written, and should assume no previous knowledge on the part of the reader.

It is perhaps inevitable that the author should emphasize eugenics and social progress at the expense of heredity; but he really overdoes it. Sexual reproduction and the chromosome cycle are once and for all described in the first four or five pages; and we then plunge into an account of Mendelism (well written and illustrated), which includes a correlation of the genetical and cytological cycles. All this occupies 30 small pages, and is much too brief and compressed to be of any but doubtful value to the lay reader; and the reviewer cannot help thinking that it is only put in as an excuse for dealing with the other subjects. These occupy four chapters, on economic applications of heredity, human heredity, population problems

and the road to social progress. These chapters are sensible and readable. The book would, in fact, be better split into two; one could then deal thoroughly with heredity and its immediate applications to domestic animals and plants, and the other with the much wider topics of population and eugenics. As it stands, the book is stimulating; and the lay reader may find the smattering of heredity sufficient to whet his taste for more.

D. H. VALENTINE.

Introduction to Floral Mechanism. By S. G. JONES. 8½ × 6 in. Pp. 274 + xi, with frontispiece, 2 plates and 71 figs. London: Blackie and Sons. 1939. 10s.

This book covers a good deal more ground than its title, and ranges pretty widely over all aspects of the flower and its sequelae. Accounts, prepared for student reading, are given of flower grouping, structure and development, the latter being treated morphologically and histologically. Pollination, fertilization and embryology follow, and then the description of fruits. A good deal of space is then devoted to heredity, its mechanism and application to plant improvement. Finally, there is classification and a detailed description of types, occupying about half the book. It is at this point that one is finally convinced that the author has attempted to serve a dual purpose. Although the title is mechanistic and the aim avowedly functional, the types considered, forty-three in number, are not related to function at all, but to a scheme of classification designed "to illustrate the method of referring a plant to its family in using a flora". This seems to mark a confusion of aim if not of thought; and it seems more than doubtful how forty-three types can be selected to illustrate adequately both the principles of floral mechanism and of angiosperm classification.

Paradoxically, a large error of treatment may be less important than small errors of statement which corrupt the foundations of the conceptual edifice. Here, however, the dispersed aim is reflected in what can only be regarded as a surprising carelessness of statement in a book intended for student consumption. No further than in the third paragraph of the first chapter we come to the consecutive sentences: "...the flowering plant proceeds to the sexual phase or formation of spores. The function of the flowers is to produce the spores, namely the pollen (male) and ovules (female); after fertilization by the pollen the ovules become seeds". According to my computation there are five misstatements in these two sentences.

The publisher's puff says that the line illustrations are of outstanding merit, and they certainly have an attractive appearance. Unfortunately, the same confusion becomes apparent immediately they are submitted to closer inspection. Even in the highly elaborated microscope studies there is nowhere an indication of the magnification used. In the macro-drawings the flowers are drawn in any position that suits the moment's fancy. The mechanism of the gorse flower depends on the weight of a bee bearing down the wings and so exploding the keel against itself. This requires a more or less horizontal placing of the flower, which is drawn cocked up towards the vertical: the piston mechanism of the lupin is similarly treated. Many of the pages are encumbered with a confusion of small and doubtfully useful drawings of isolated floral parts. Some of them are also inconsistent. Fig. 66 (bluebell) shows a posterior loculus in the upside-down drawing of the half-flower; but, in the floral diagram alongside, this position is occupied by a septum. The same inconsistency appears in the next figure (jonquil) also. In Fig. 57 (white dead nettle) the representations of the ovary are in disagreement again; the median elevation suggests an anterior sepal by gross exaggeration of the tube and is doubtful about the existence of a posterior one. The hood is so out of proportion as to have no overhanging horizontal limb at all; a malformation which I have been unable to find in any one of a large number of flowers examined in the Oxford district. The floral diagram has no indication of the fission of the ovary, although it is shown in the sectional drawing. This is perhaps rather more than the usual number of inconsistencies on a single page, but it by no means exhausts the list.

It is much to be regretted that a book, which on account of its general attractiveness is sure to be widely used, should contain such unfortunate pitfalls for the young student.

W. O. JAMES